A STAFF COLLEGE FOR TECHNICAL TEACHERS

IT is fitting that, in what is likely to be his last public pronouncement as chairman of Britain's largest limited liability company, Sir Alexander Fleck should be identified with an appeal to set up a staff college for technical teachers. It was in the evening classes of a technical college that, many years ago, Fleck began a career as a student chemist which was later to take him to fellowship of the Royal Society and the chief executive's position in Imperial Chemical Industries, Ltd.

The idea of a staff college for teachers in technical institutions in Britain was first mooted by the Education Committee of the Federation of British Industries in 1956. It had grown out of the experience of members of the Committee who belonged to large industrial undertakings and whose companies had found it advantageous to establish a centre where they could send senior staff for a short intensive course designed to enlarge their personal qualities and ability. The idea was taken up afterwards by the Special Committee on the Supply and Training of Teachers for Technical Colleges appointed by the Minister of Education, of which Sir Willis Jackson was chairman, and it was discussed in the Committee's report*. This recommendation was widely supported by many authorities, including the Minister of Education's own Advisory Councils on Education for Industry and Commerce, and for the Training and Supply of Teachers. The latter Council, at the Minister's request, made a more detailed study of the proposal through a new sub-committee, of which Sir Willis Jackson was again chairman.

The place of the staff college as a means of developing quality in advanced technical education has been well put in a memorandum prepared by this sub-committee (not published). At the top level of the technical teaching profession there is a need for men with the breadth of outlook, attitude of mind and authority required to stimulate up-to-date teaching and research work of high standard, to talk with conviction to executives of industry and to exert an influence on thought and action outside as well as inside the colleges. Existing facilities for teacher training, or for keeping teachers in touch with new developments by means of short courses, do not provide the right kind of experience for senior staff; and the normal contacts which a teacher makes with local industry through advisory committees, and the day-to-day operation of technical courses, do not bring him into close enough contact with a sufficiently wide range of industry.

To fill the gap, a national centre is needed where carefully selected senior staff from the colleges can exchange information, ideas and experience, by direct contact and personal discussion, with each other and with personnel from industry, the universities and associated fields. Experience with staff colleges organized by industry has already shown that this function can be discharged most effectively within small groups working in close personal association.

It is not only senior teachers in technical colleges who would benefit from the establishment of the proposed staff college. Industry would benefit indirectly through the improved quality of technical education which should result, but could also benefit directly through participation in courses by those responsible for training schemes in industry or for co-operation with technical colleges on research projects.

The staff college envisaged by the Willis Jackson sub-committee would be a small establishment providing a series of short courses throughout the year under the general supervision of a permanent director of studies. The courses would be limited to about twenty teachers, together with about half that number of individuals drawn from industry and commerce. As there are about 17,000 full-time teachers in technical colleges in Britain, those selected to attend would probably belong to the 3,300 or so among that number who are of the grade of senior lecturer or higher. Probably not more than about 200 teachers a year would pass through the college.

What could such a college achieve? Its main object would be to ensure an adequate supply of key personnel for the technical colleges—the future principals, heads of departments and other key staff. Ultimately, success of the Government's policy for expanding facilities in technical colleges, for which the sum so far authorized is about £150 millions, will depend upon these men. Already well-versed in some particular branch of technology, good teachers, and experienced administrators, they would be given the opportunity of discovering in themselves further qualities of leadership and authority.

Such a college would not strictly be regarded as a teaching institution. The composition of a group would vary according to the subject of the course. There would be no 'instruction' in the ordinary sense. The participants would jointly study some main problem or topic in the field of technology, with particular reference to its significance for teaching and administration in the technical colleges. The members of a group would be their own teachers in a co-operative study to which each individual would make a reponsible contribution. For this reason, the number of the directing staff would be kept as low as

* Ministry of Education. The Supply and Training of Teachers for Technical Colleges: Report of a Special Committee appointed by the Minister of Education in September 1956. Pp. xiv+68. (London: H.M. Stationery Office, 1957.) 4s. net.

possible, but they would have to be of very high quality.

A remarkable and highly commendable feature of the way in which the proposal for a staff college has developed is that it can truly be called a co-operative venture between industry and education. The idea originally came from industrial representatives; it was taken up and developed by a committee set up by the Ministry of Education. An approach by industry was therefore made to the Ministry about the way such a college could be financed. The Ministry of Education consulted local education authorities, and the scheme put forward by Sir Alexander Fleck can now command the support of industry, central and local government.

To establish such a college an initial sum of £100,000 will be required. The annual running cost would be about £30,000. Exchanges between Sir Alexander Fleck and the Minister of Education have revealed that, if industry would find the original £100,000, the running costs would largely be met from public funds. To keep fees for courses down to a reasonable figure, the Ministry itself is prepared to pay an annual grant of up to £10,000 to the college. This subvention, amounting to about one-third of the running cost, should enable the college to offer its facilities for a fee of about £75 for a three-week course. For the most part these fees will be paid by local education authorities as the employers of the teachers attending the course. The remainder would come from fees paid by the employers of other participants, including industrial and commercial firms.

In private approaches to some thirty firms, Sir Alexander has already been promised £60,000 of the capital cost. He, and the industrial sponsors, have shown their wisdom by making a public appeal to any firms which care to participate. If all the money had been collected from a comparatively few large firms, the college would have got off to a bad start. From the beginning it should be regarded as one in which all industrial organizations in Britain have had the opportunity to participate; it is to be hoped that many small firms will contribute.

It is not only in finance that the proposed college can be regarded as a joint enterprise between education and industry. Sir David Eccles has discussed the proposals in general with the associations of local authorities and agreed that, on the governing body, there should be equal representation of local authorities, technical teachers and industry and commerce, together with a university representative. What is even more noteworthy is that the Minister has already asked Sir Alexander to accept the chairmanship of this non-existent governing body and that Sir Alexander has accepted.

Apart from the intrinsic merits of the proposal to set up this staff college, a number of features commend themselves. First, it is an excellent example of the way in which industry and education should come together to promote the development of technological education. Secondly, it shows that a Government department can act quickly when the right approach is made to it by industry. Thirdly, the small group of men, who first put forward the idea some four to five years ago and met a stone wall, hard and apparently unclimbable, may now rejoice that the first courses in the new college should take place before the end of 1961. There is little doubt that such a college will be the centre of much interest, not least among those responsible for the administration of universities.

ROCKET PROPULSION

Rocket Propulsion

By Marcel Barrère, Prof. André Jaumotte, Prof. Baudouin Fraeijs de Veubeke and Jean Vandenkerckhove. Pp. xxxi+829. (Amsterdam: Elsevier Publishing Company; London: D. Van Nostrand Company, Ltd., 1960.) 135*s*.

A GOOD book on rocket propulsion has long been awaited, and I received this one with hope. I regret to say that my hopes have not been realized, and this is all the more disappointing because of the standing of the four authors. It would have been enlightening to know the type of reader whom the authors had in mind when compiling this book. It is not satisfactory as a text-book for students, who require instruction in basic principles and only sufficient technology to show how principles are applied, nor is it satisfactory for people actually engaged in rocket design and development. In this latter respect it is perhaps doubtful whether a good book on rocket technology can yet be written because the predominantly military application of rockets brings in its train security difficulties. The net result is that the 'technology' which can be revealed is either obsolete, or even misleading, and in this context it must be realized that something done two years ago may be obsolete.

Reverting to the possibility of regarding this book as a basic work for students, it fails in many respects, and the text contains much irrelevant matter which I found distracting, nay confusing.

Chapter 1, "Rocket Propulsion Elements", scarcely lives up to its name. "Specific Impulse", that all-important criterion of rocket performance, receives brief initial mention on p. 33, surrounded by trivia on "Useful Power" and "Propulsive Efficiency". Surely the latter part of this chapter headed "Nuclear Energy" is nothing but misplaced padding, but a more serious criticism relates to the section of this chapter headed "Conclusion". A completely unjustifiable assumption relating a portion of the rocket weight to specific impulse is made on p. 52, leading, as one might expect, to a completely erroneous conclusion regarding optimum specific impulse: a sad beginning to more disappointments.

Chapter 2 has the title "Nozzle Theory and Characteristic Parameters". Perhaps we can forgive the misuse of the word 'parameter', which occurs frequently throughout the book, but this chapter occupies 71 pages, yet the authors state in their second paragraph, "Whilst refraining from going into detailed theory of nozzles, we shall summarize the principal relationships needed for our purpose". Certainly they refrain from devoting just one page to derive the expression:

$$\frac{dA}{A} + \frac{dv}{v}\left[1 - \frac{v^2}{a^2}\right] = 0$$

where A is the cross-sectional area of the nozzle; v is the corresponding velocity of the gases in the nozzle; a is the local velocity of sound, from which the convergent–divergent nozzle 'emerges'. Its introduction might have saved the authors twenty pages of print and given the reader a true appreciation of the Laval nozzle.

So the text goes on for a total of 811 pages with much that is of little positive value. Very few of the basic conclusions are derived, and on many occasions important statements are made for which one would rightly expect some proof. But, no. Surely one is justified in expecting a short proof that if n is the index of the burning pressure, governing the burning-rate of solid propellents, equilibrium can be established only if n is less than unity. Incidentally, on p. 240 one reads that if n is greater than unity an explosion will occur. In fact, if n is equal to unity an explosion will occur—a slight but most important difference. Erosive burning is referred to on pp. 201 and 202 and again on pp. 259 and 260, and the two references show little connexion with each other. Why could not the authors deal with it once, and once only?

Many examples of wrong statements may be quoted and one or two will be sufficient to indicate the worries created in my mind. On p. 317 we find "The nozzle weight is found to be approximately proportional to total impulse". This is just not true, and there is no reason why it should be. Yet again, in section 7.2.5, "Geometry of the Combustion Chamber", one reads that there are 'trends' in combustion chamber shape and infers that they are subjected to the same whims of fashion as ladies' waists and hem-lines. If this were true, why did the authors devote 100 pages and more in Chapter 7 to the problems of liquid-propellent rocket combustion chambers and another 60 pages in chapter 10 to combustion instability? Yet, throughout, the authors make implicit assumptions on chamber shape. On p. 468 we read that "the Mach number in the chamber is low, being of the order of 0·1, we can neglect its influence". Thus, whether the authors realized it or not, they are implying that the ratio:

$$\frac{\text{combustion chamber cross-sectional area}}{\text{nozzle throat area}}$$

is greater than 5 : 1. To say the least, this is at variance with earlier remarks on 'trends'.

Occasionally we get glimpses of the true position in rocket research and development as in paragraph 10.4 on p. 707. "A change in the scale can completely alter the stability conditions of the power plant, and it is difficult to predict the operating stability of a large rocket on the basis of experimental results obtained on small-scale models." I certainly accept this as true and regard it as an incentive to do some basic work to rectify the position. The book takes 50 pages to say the same thing.

To anyone diligent enough to pursue some of the numerous references given at the end of each chapter there is a more enlightened story awaiting them. It is noteworthy that most of these references refer to American documents, and the authors can scarcely be blamed for the paucity of references to British work.

After 'scanning' this book, I am a sadder, but no wiser, man.

J. E. P. Dunning

NEUTRON PHYSICS

Introduction to Neutron Physics

By L. F. Curtiss. (Van Nostrand Nuclear Science Series.) Pp. xi+380. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1959.) 73*s*.

THERE is now an extensive literature on neutron interactions and on the techniques used in their study. A student starting work in this field is frequently confused by the proliferation of original papers, and needs a text-book which will give him a grounding in this subject of neutron physics. It is the aim of Dr. Curtiss's book to do just this; and on the whole it succeeds.

The emphasis of the book is on experimentally demonstrated facts and widely used techniques. The level of presentation is fairly elementary, but the book is easy to read and the style is clear and flowing. A student should have no difficulty in mastering the text and doing the problems which are found at the ends of the chapters. There is a lot of simple details which will help to give the student a 'feel' for this subject, but there is little critical discussion which would lead the student into the more complex parts of neutron physics.

In writing a book of this type it is often difficult to keep it up to date. This review will appear some eighteen months after the author wrote his preface, but from an examination of the text and the references it seems that the literature is covered up to 1956 only, so that the book is effectively four years old already. Of course all authors and publishers are familiar with this problem, and to counteract it some type of general bibliography covering the last few years might well have been included during the final preparation. In spite of this defect, this book covers all the groundwork of its subject.

Although most branches of neutron physics receive attention, there are occasional lapses. For example, the linear electron accelerator is not mentioned in the discussion of pulsed neutron sources; and inelastic scattering of thermal neutrons is entirely omitted. In discussing thermal neutron interactions Dr. Curtiss uses the term 'diffuse' cross-section in place of the now almost universally used term 'incoherent' cross-section, which may confuse some of his readers.

Nevertheless this book will command a wide audience and will be found both necessary and useful in many laboratories. The presentation is good, the diagrams are clear and there are very few misprints. The author and the publishers are to be congratulated on the result of their labours.

P. A. Egelstaff

INSECT PHOTOGRAPHY

Les Plus Beaux Insectes

Textes de A. Villiers. (Collection "Nature et Beauté".) Pp. 96 (154 illustrations). (Paris: Larousse, 1958.) n.p.

THIS quarto volume is one of the Larousse series "Nature et Beauté", and is primarily an atlas of more than one hundred and fifty photographs of insects. These are from a variety of sources, some showing insects in natural, or quasi-natural settings, others from pinned and set specimens, formally arranged into a plate. Twenty-four full-page plates are in colour, printed on an art paper with a lustre

surface which is pleasant to the touch, and which displays the colours well, without any troublesome reflexions.

The reproduction of colours is good for the browns and yellows. The Orthoptera in Figs. 31*a*, 31*b* and the Hymenoptera in Figs. 63*a*, 63*b* are full of life, as well as structural detail. The reds are much less strident than in some French colour-plates of insects. Least successful colours are the pastel blues and greens, so that *Agrion splendens* belies its name in Fig. 7, and the long-horned grasshopper in Fig. 26 has a ghostly appearance. Definition and reproduction of detail are excellent in nearly all plates, black-and-white as well as colour.

The captions usually give the natural size of the insect, but it is to be regretted that quite a number of them wrongly identify the insects shown. Sometimes the whole caption is inappropriate, as if another photograph had been substituted for the original. Figs. 44*a*, *b*, *c* have clearly been re-arranged for artistic effect, leaving each of them with the wrong caption. This trouble seems to apply particularly to the Diptera, and it is unfortunate that the very first page of text is accompanied by a large head of a female *Tabanus* labelled "Gasterophilus equi".

The text is more extensive than in many pictorial works of this kind, and is quite detailed enough for the general reader. Occasionally it descends to such banalities as: "La ponte des Hémiptères n'est pas très originale: . . .".

Apart from the incorrect captions, this is altogether a pleasant work to possess. Since no price is quoted it cannot be said whether or not it is good value for money.

H. Oldroyd

MEYRICK'S MICROLEPIDOPTERA

Catalogue of the Type Specimens of Microlepidoptera in the British Museum (Natural History) described by Edward Meyrick

By J. F. Gates Clarke. Vol. 3: Tortricidae; Olethreutidae; Noctuidae. Pp. iv+600. (London: British Museum (Natural History), 1958.) 120*s*.

THIS, the third volume of a planned series of six or seven, is the second of those devoted to illustrating the type-specimens of species described by Meyrick. It deals with some 380 species of Tortricidæ, and 567 of Olethreutidæ, while the inclusion of a single noctuid results from an original misidentification at family level. A prefatory note states that several old names of long-standing use have been dropped. As a result of this and other redispositions new combinations abound, notably in *Acleris*, *Archips* and *Olethreutes*. It is explicitly stated, however, that the new combinations do not necessarily represent a definitive evaluation; but there is no indication which combinations have such provisional status.

In each family the arrangement is alphabetical, both for genera and species, with cross-references to specific synonymy but not to intergeneric transfers. The latter omission is apt to occasion the reader a long search if he should fail to locate a species in its customary genus. The text referring to each species gives the original bibliographical reference, and the wing-span and locality of capture of the type, the number and sexes of the original series, an indication or designation of the type specimen, and an explanation of the accompanying illustrations. Comment is sparse and usually restricted to correction of errors or clarification of obscurities. The 298 plates illustrate photographically the fore- and hind-wing of each species, the genitalia of most and the labial palpi of some. Where the type is the basis of a genus, a line illustration of the wing venation is also given.

The task of presenting taxonomically significant data by means of photographs has been successfully accomplished (though some of the genitalic photographs are barely free from ambiguity) and the uniform arrangement of the illustrations makes the plates satisfying as well as informative. So far as possible, the illustrations have been arranged to face the text to which they refer, a real convenience which justifies an occasional unwelcome displacement of species. The volume, like its immediate predecessor, gives enough data to permit identification of almost all the species discussed, and will be a mine of source data in revisionary work.

Considered from the point of view of a pioneering work in dissemination of critical data on type specimens, this series may well form a pattern for later catalogues of Lepidoptera; but it is to be feared that in comparatively few other groups of insects will photographic representation meet the standard of illustration required.

R. G. Fennah

PROBLEMS OF HUMAN FERTILITY

Modern Science and the Human Fertility Problem

By Richard L. Meier. Pp. xiii+263. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959. Sponsored by the Conservation Foundation, New York.) 48*s*. net.

HERE is a book which was badly needed. It deals with one of the most urgent of the many problems facing mankind at the present time—the menacing increase in the world population. In an early chapter entitled "Equilibrium in Human Population", the author states that "the foreseeable limit is set by the quantity of energy that is steadily reaching us from the Sun". It depends also upon an estimate of the minimum human (*per capita*) "requirements for energy in efficiently organized civilizations". The present rate of population increase works out at 1·6 per cent, and the author estimates that the limits of continuous energy supply may be overtaxed by A.D. 2100, a date which is only five or six generations ahead of us from now.

Dr. Meier's review of the situation is the completest that I have yet come across. In an early chapter he examines the population problem on a miniature scale as it exists in the island of Puerto Rico, where the general population density now exceeds that of any European country. He made a personal study of the situation among the poorer families and also the attitude of the Puerto Ricans to attempts of the authorities to introduce contraceptive measures. He describes the unexpected popularity of surgical sterilization of overburdened mothers, and the reactions of the various religious denominations to birth-control measures in general. Finally he tells us that the situation has been prevented from becoming critical in Costa Rica by a continuous overflow of the population on to the mainland. The author also made a similar study of the problem in the over-populated island of Mauritius.

Later chapters of his book deal with the future measures which will be required for staving off disaster, and the discovery of an ideal method of contraception is the most pressing need of all. All experts on this subject agree that what is required is a preparation which can be taken by the mouth and which is guaranteed to cause no harm. This pill may work in various ways ; by inhibiting ovulation or the nidation and the development of the fertilized ovum ; or by inhibiting spermatogenesis in the male. But there are other links in the chain of fecundation which might be broken artificially and these possibilities are reviewed by the author in an appendix. He rightly deplores the fact that whereas immense sums of money can easily be raised for research on a cure for cancer, there is little financial support for what is a far more urgent human problem. But Dr. Meier does not despair. He believes that the situation "is such that we must not reasonably expect to find that the first mass manufacture and use of oral contraception will come into being before the 1970–75 period".

Dr. Meier's book is the completest and the best survey of the world population problem which has yet appeared, and it is a work which is indispensable to everybody who is interested in this all-important subject.

KENNETH WALKER

TEXTILE ENGINEERING

Textile Engineering Processes

Edited by Prof. A. H. Nissan. Pp. ix + 366. (London : Butterworths Scientific Publications ; New York : Academic Press, Inc., 1959.) 60*s.* ; 9.25 dollars.

MODERN textile industry is becoming increasingly dependent for its future prosperity on the recruitment of graduate scientists and engineers, so that it can derive the greatest benefit from modern scientific discoveries arising from research and development into textile materials and processing. Several books are available in which the chemical and physical aspects of textiles are discussed, but little has been written with the needs of engineers in mind. Such a book has become more necessary now that the Institution of Mechanical Engineers has created an option in textile engineering in the examination for the associate membership.

The present volume is intended to give to engineers a concise account of the processes involved in converting fibres into fabrics, special attention being paid to the mechanical aspects of the subject. Difficulties of writing such a work are considerable, for the textile industry is divided into many specialized sections. Prof. Nissan has, however, been successful in obtaining several collaborators, each of whom is a specialist in his own field. It is impossible to survey all the important aspects of textiles in the space available, and consequently attention has been limited to the following topics : physical properties of textile fibres, yarns and fabrics ; the principles and techniques of yarn and cloth production, including knitting ; wet finishing, and dyeing and printing machinery. Rather more specialized accounts of the important topics of drying, humidity control, and automatic control complete the volume. Each chapter is in effect an essay on a particular aspect of textile processing. The various authors appear to have interpreted their terms of reference rather differently, but all have given clear and concise accounts of their subject. Some have treated topics in a general descriptive way while others have chosen to discuss specific aspects in greater detail. These differences are offset to some extent by the suggestions for further reading given at the end of each chapter.

The book is a welcome addition to the literature on textiles. As an introduction to the subject, engineers and physicists will find it useful particularly in its treatment of yarn and cloth manufacture, while those already acquainted with textiles will appreciate the parts dealing with more specialized topics. It is unfortunate that the book is so expensive ; if the present trend in the price of text-books continues, students will soon be unable to buy them.

C. S. WHEWELL

BONDS BETWEEN RUBBER AND METAL

Rubber to Metal Bonding

By Dr. S. Buchan. Second edition, revised. Pp. xiv + 300 + 35 plates. (London : Crosby Lockwood and Son, Ltd., 1959.) 42*s.* net.

THE first edition of Dr. Buchan's book, published in 1948, described the technological situation as it existed after the intense effort of the War. Most of it was concerned with the very successful brass plating method of achieving bonds between rubber and metal, and Buchan's own work, aimed at understanding the mechanism as well as rigidly controlling the product, was naturally described in great detail. The evidence he presented pointed strongly to a sulphur bond between the copper of α-brass and the rubber. A further ten years have passed ; but our knowledge remains where it was, and one is led to question the usefulness of printing a second edition. True, the revision has been thorough, as all chapters bear evidence by the addition of more recent information from the literature and from the author's experience with the newer chemical bonding agents, but the scientific situation remains unaltered and the chapter on the nature of the rubber-to-metal bond, although rewritten, is weak.

Technologically, the book is still comprehensive and indispensable because it gives practical details, formulæ and, most important, the test results obtained. Some processes, such as latex-blood albumen, are now obsolete and could have been deleted, while the space devoted to the brass-plating method, although valuable, would give the newcomer a false idea of its present popularity. The reviewer knows of no new plants for producing bonded rubbers by this method, while some have actually closed down or retracted in scale. The new chapters on chemical methods added to the second edition do not make this point nor do they give any indication of comparative processing or capital costs. Is it just this factor that has told against the brass-plating process ?

There are a few minor blemishes for which the publisher must be blamed. A large proportion, in fact nearly all, of the graphs shown are not professionally drawn, and the lettering is bad. 'm.m.' for 'mm' is irritating as well as wrong. 'G.R.S.' for 'GR-S' is inelegant and in any event it was superseded during 1957 by 'SBR', which is now universally used as the abbreviation for butadiene-styrene rubber.

W. C. WAKE

The Morphology and Anatomy of American Species of the Genus *Psaronius*

By Jeanne Morgan. (Illinois Biological Monographs, No. 27.) Pp. vii+108. (Urbana, Ill.: University of Illinois Press, 1959.) Paperbound, 3 dollars; Clothbound, 4 dollars.

THE name *Psaronius* is applied to certain fossil tree-fern stems of Permian and Carboniferous age. The author in this monographic treatment of the American *Psaronii* describes three new species of Pennsylvanian Age from the United States and suggests that there are in all six valid species of the genus in America. From a careful study of about twenty-three specimens she has been able to allocate them to the three new species. There is considerable variation among the specimens allocated to any one species; but this variation, which is principally manifested in varying complexity in the polycyclic stelar anatomy, is due, she assumes, to ontogenetic variation such as is found in existing tree-ferns where the relatively small and simple stelar arrangements at the base of the tree formed in its sporeling state are succeeded higher up by increasingly complex cyclic polystelic arrangement. Some corroborative evidence in support of this assumption is obtained from specimens in which a considerable length of a stem has been found. She gives an account of the complex relationship between the several cycles of meristeles and the leaf-trace system connected to it. She also shows how the phyllotaxy may change between the base and upper parts of a stem, new orthostichies being intercalated and sometimes a spiral phyllotaxy is replaced higher up by a whorled arrangement of leaves. In places her descriptions are difficult to follow; a greater use of lettering on the diagrams would have helped the reader to follow her arguments. She places these fossils in a separate natural order, Psaroniaceae, although a good deal of evidence supports the view that they are related to the Marattiaceae. It is, however, a very useful contribution to our knowledge and is well illustrated and adequately indexed. JOHN WALTON

Astronomical Photography at the Telescope

By Thomas Rackham. Pp. 232+11 plates. (London: Faber and Faber, Ltd., 1959.) 36*s*. net.

MOST popular works on astronomy are illustrated by a series of impressive photographs taken with the world's largest telescopes. The casual reader may therefore get the impression that the photographs taken with smaller telescopes of the size owned by the average amateur astronomer are correspondingly poor and uninteresting. However, this is not necessarily the case, and in this book Mr. Rackham explains to the reader the techniques which he has perfected by many hours at the telescope. After clearing the ground with a lucid account of the basic photographic process, he proceeds to describe in detail the instruments and accessories used—nearly all capable of construction by the intelligent layman. The most common subjects of photography for the amateur astronomer are the Sun, Moon, Mars, and Jupiter, of which the individual difficulties are discussed at length. The photography of star fields, either through a telescope or by means of an ordinary camera, is not treated so fully; for this the reader is referred to King's "Manual of Celestial Photography". The chapters on practical processing and darkroom technique should answer all conceivable queries, although the professional astronomer will be surprised to find film almost invariably used instead of plates.

In conclusion, the book can be best summarized in the author's own words—"The amateur's work may not always be of scientific value . . . but it will introduce him to the many wonders of astronomy where they are properly to be found—not in books, but in the sky". D. H. P. JONES

Physical Geography

By Philip Lake. Fourth edition, edited by J. A. Steers. Pp. xxviii+483+25 plates. (Cambridge: At the University Press, 1958.) 25*s*.

PHILIP LAKE'S "Physical Geography" was first published in 1915 and so quickly established itself as to be reprinted a dozen times before the author's death in 1949. It was in that year that the second edition appeared, re-written rather than revised by a team of Cambridge geographers under the leadership of J. A. Steers. Whether or not one accepts the definition of geography as human ecology it will be universally agreed that a proper understanding of man's environment demands a knowledge—it may be general but must be soundly based—of the physical factors of that environment. The successive editions of Lake have sought to incorporate the results of work in the now numerous related fields of study. To the old three-fold division into the atmosphere, the ocean and the land, still preserving such empirical chapters as 'coral reefs', 'volcanoes' and 'lakes', have been added in this latest edition three more chapters on floods and storms—a by-product of Prof. Steers's special concern with the East Coast floods of 1953—on soils by A. T. Grove, which brings in some, but not very much ecology, and on marine life by A. J. Lee. It has sometimes been said that a student entering the Cambridge School of Geography will enjoy excellent series of lectures by specialists but will be left to discover for himself the integrated whole which modern geography should be. Something of the same criticism applies to the latest edition of Lake's "Physical Geography".

L. DUDLEY STAMP

Classification and Indexing in Science

By B. C. Vickery and D. J. Foskett. Second edition, enlarged. Pp. xix+235. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 30*s*.; 6 dollars.

THE second edition of this book has been considerably expanded without affecting its character as a study of method rather than a manual of classification and indexing. The first three chapters and appendixes are almost unaltered apart from some transpositions in the appendix dealing with historical aspects and an extended treatment of the Universal Decimal Classification. Those on "Indexing" and on "Classification and Mechanical Selection" have been transposed: the former now includes a brief section discussing the indexing of chemical compounds and the latter some account of mechanisms used for retrieving information and examples of the subject analysis and coding used. The bibliographies of these two chapters and also of the following chapter "The Future of Information Retrieved", which has been completely re-written to take account of developments reported to the International Conference on Scientific Information at Washington in November 1958, have been expanded. R. BRIGHTMAN

THE ROYAL SOCIETY'S EXPEDITION TO HALLEY BAY DURING THE INTERNATIONAL GEOPHYSICAL YEAR

ON February 4 the Royal Society spent a day listening to accounts by various members of the expedition to Halley Bay of the work done in Antarctica by the British team during the International Geophysical Year.

The discussion was led by Sir Graham Sutton, who began by recalling that although a proposal to mount an international expedition to explore the Antarctic continent had been made as long ago as 1861, it was not until the present century that any significant progress was made. The initial phase, of wooden ships and animal or man-drawn sledges, culminated in the large expeditions of 1910–15, and although conditions are vastly different to-day, there are still hardships and even perils to be faced. The choice of a site in the Weddell Sea area was dictated by a number of factors; but there is no doubt that, from the geophysical aspect, the area is of great interest.

Mr. J. MacDowall (who was the leader of the expedition in 1958) opened the discussion with an account of the work done in seismology, glaciology and meteorology. The observatory was built on a floating ice shelf 29 m. above sea-level in latitude 75° 31′ S., longitude 26° 37′ W. Considerable microseismic activity was observed during the summer (December–February), especially with on-shore winds, and he gave examples to show the relation between microseisms and wind force. The short-period three-component Willmore seismograph used on the floating ice shelf was found to detect *P*-waves at most epicentral distances, but *S*-waves were badly recorded. The shelf itself had a yearly addition of about 1 m. of snow of mean density 0·36 gm. cm.$^{-3}$. Considerable ablation occurred, even in winter, and it is not easy to account for this in terms of the energy balance.

Mr. MacDowall directed attention to the small range of monthly mean air-temperature through the troposphere compared with the very large difference in extremes in the stratosphere, where the records indicate a range of 59° C. at the 30-mb. level. Another interesting fact is that there is a notable difference between the northern and southern hemispheres in the seasonal change in atmospheric ozone. The spectrophotometer measurements of the total amount of ozone made at Halley Bay during 1956–58 do not appear to fit into the hitherto accepted global pattern of seasonal and latitudinal variations, and only the short-period fluctuations were found to be related to upper-air measurements. In south-east England both the long- and the short-period variations are related.

Mr. J. M. C. Burton described observations on the nature of the ice shelf and drew the conclusion that it is partially grounded to the east-north-east, but floating to the south. He suggested that the Dawson Lambton glacier should be taken as the true coast-line. The Allan MacDonald glacier, which Shackleton reported in 1914 some 30 miles north of the Dawson Lambton glacier, was not seen during the two years of the International Geophysical Year, and he regarded its existence as doubtful.

Commenting on these contributions, Dr. R. C. Sutcliffe dealt with the ozone distribution and suggested a possible explanation in terms of a thermal circulation. Mr. H. H. Lamb continued with an analysis of upper-air measurements. He pointed out that it is only since 1957 that any general picture of stratospheric circulation over the Antarctic can be constructed, and of the three years in question, it seems fairly certain that 1959 was anomalous in the late winter and spring in regard to winds, temperatures and maximum amounts of ozone in the stratosphere.

Mr. W. H. Bellchambers and Mr. W. R. Piggott described the ionospheric measurements. The Halley Bay observatory, being near the centre of the Weddell Sea geomagnetic anomaly and yet sufficiently outside the auroral zone to avoid the effects of minor corpuscular magnetic or auroral activity, is particularly well placed for the study of the electrified part of the atmosphere. The main conclusions reached are that the *F*2-layer variations are dominated by movements of ionization (which can also be seen near the maximum of the *F*1-layer), and that ionization drift velocities in the *E*-region reverse with season and are greater in winter than summer. The incidence of 'spread *F*' is very anomalous, being essentially a magnetically 'quiet day' phenomenon.

In the general discussion, Dr. R. L. Smith-Rose, after paying tribute to the excellent work done by the team, remarked that two features of the results were surprising. In the temperate and auroral latitudes of the northern hemisphere severe 'spread *F*' is closely correlated with magnetic and auroral activity and is well known as a precursor to the failure of trans-Atlantic radio communication. At Halley Bay, on the other hand, it seems to be associated with relatively quiet conditions. Again, in the northern hemisphere the maxima and minima of *foF*2 are approximately at the same local mean time at most ionospheric stations. This does not suggest the type of movements proposed by the speakers. He asked if there is evidence for any fundamental difference in the behaviour of the layer in the two hemispheres.

Mr. P. M. Brenan and Mr. D. P. Harrison gave an account of the work in radio astronomy. It was found that ionospheric velocities, deduced from the motion of the diffraction pattern on the ground, were approximately towards the west in the first part of the night and towards the east for the remainder of the night and most of the following day, the reversals taking place about 0030 and 1630 L.M.T. The speeds were of the order of 400 m.sec.$^{-1}$ in the evening, falling to about 100 m.sec.$^{-1}$ in the easterly motion. The times of change and the magnitude of the drift speeds agree with measurements of the motions of auroral forms made at Halley Bay by S. Evans (1959) using an all-sky camera.

For radio-echo observations of auroræ and meteors, a low-power equipment working with a rotating aerial on a frequency of 71·3 mc./s. was used, similar to that at Jodrell Bank. The auroral echoes fit

closely the case of specular reflexion for columns of ionization aligned along the local magnetic field at about 100 km.

Commenting on these papers, Dr. J. G. Davies remarked that a clear correlation between the peaks of auroral activity at Halley Bay and the rarer auroral echoes at Jodrell Bank has been demonstrated. The scattering mechanism is clearly specular. He pointed out that in the auroral case, as well as for the work on radio-source scintillations, the fact that the observations appear to be in regions of increased ionization elongated along the direction of the local magnetic field suggests that these may be extensions of the van Allen radiation belts, and may represent the mechanism whereby the radiation zones drain into the atmosphere.

Dr. S. Evans and Dr. G. M. Thomas gave an account of the visual work on aurora. An all-sky camera was used. A plot of the hourly-mean vector movement indicated a systematic diurnal component, reaching a few hundred metres a second in the easterly and westerly directions. Comparison with the horizontal magnetic disturbance vector averaged over the same period was held by Dr. Evans to imply an effective transport of negative charge by aurora.

Dr. Thomas said that a feature of the auroral display at Halley Bay was the almost continuous presence of a quiet arc lying well to the south. There is evidence, supported by observations in other localities, that there is a migration of quiet arcs to higher latitudes at times of maximum sunspot activity.

Mr. J. Paton, discussing these results, speculated whether the small movement in the position of the quiet arcs recorded at Halley Bay between 1956 and 1958, if it is real, is related to changes in the form of the outer van Allen radiation belt during the period between sunspot minimum and maximum. He also asked if the observations now indicate that the phenomena of 'flaming' occur only on the equatorial side of the auroral zones, and that the somewhat surprising rarity of red coloration in the Halley Bay displays indicates the general rule that persistent red diffuse surfaces are very limited in their geographical position, being virtually confined to the middle latitudes.

Sir Graham Sutton, summing up, said that, in his opinion, the Society had been amply justified in its decision to send an expedition to this remote part. The data obtained seemed to be unique in many ways.

The president of the Royal Society, Sir Cyril Hinshelwood, in closing the meeting, paid tribute to all who had taken part, and said he had never had any doubt that the Halley Bay venture would be worth while. From what he had heard, the geophysical services would benefit greatly.

The contributions and a report of the discussion will appear in a forthcoming number of the *Proceedings of the Royal Society*. O. G. Sutton

THE TEACHING OF VETERINARY ANATOMY AS A BRANCH OF BIOLOGY

ITS RELATIONSHIPS TO HUMAN ANATOMY

By Dr. R. A. GREEN

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EVERYONE has the opportunity of gaining inside knowledge in one only of the sciences—biology. He carries around with him until he dies a living specimen about which he can know something at first hand. This intimate knowledge of vital activity is not only unique, it also carries with it a greater depth of understanding than is possible by the use of the intellect alone. Certain branches of knowledge like mathematics claim to depend on the use of pure reason. Any branch of the natural sciences, however, makes use to some extent of man's knowledge of his own body.

Anatomy is fundamentally a branch of biology but, based historically on cadavers and the descriptive method, it is often thought of as a dead science—in more ways than one. It is also mistakenly considered to be a highly concrete science because it deals with the gross aspects of structure which are not only three-dimensionally solid but which can also be prodded with the fingers. To bring the dead organs to life, an older generation therefore added 'function' as something over and against 'form'. Thus was born one of the many antitheses that used to divide biologists into two warring camps. Nowadays they are mostly too busy being experimentalists to worry about these many unsolved problems, and this is no doubt why words like 'teleological' and 'Lamarckistic' are used less often as epithets of derision.

In fact, as Woodger first remarked[1], the antithesis of form and function is readily resolved in terms of ideas on space/time. A dead organ, far from being one of the most concrete elements in biology, is one of the most abstract. It is a four-dimensional object, from which the dimension of time has been removed. Living structure, by its very nature, has a history. It is changing from moment to moment. Form and function are but two aspects of a single entity—a living organism. To consider any living organism from one only of these two aspects is a highly artificial abstraction from reality. Similarly, because the organism is a structuro-functional whole, considerations in isolation of one of its several organ-systems or of separate organs are still further stages in abstraction, invented by the mind of man. Thus, living structure *is* function.

For some years now in the teaching of human anatomy there has been a tendency to depart from the older descriptive method of dealing with gross structure. Function is introduced in many places, and organs are dealt with in terms of the functions which they are designed to carry out in life. This is

occasionally an overtly teleological approach which is all to the good; for, as MacDougall once said[1], there are quite clearly two kinds of causation at large in the world—mechanical and teleological—and, of these, teleological causation is fundamental. Man conceived the idea of inanimate objects pushing each other about because of what he knew at first hand of the results of using his own muscular energy; and, in as much as events happen in the realm of living things *as though* those events were purposive[2]—it does not seem a serious sin to treat them that way if to do so is to further understanding. To hold the opposite view, that in order to be scientific, any statement concerning living things and how they act or are made must be based on a materialistic philosophy, is merely to display a narrow dogmatism that in no way accords with the facts of observation[3].

There was a time when observation played a prime part in the biological sciences. From it was born the descriptive style of anatomy; but like any thought-method indulged in as a specialization this eventually began to defeat itself[4]. The sheer accumulation of descriptive facts began to have an arid effect and became intellectually unsatisfying. Certain details began to be relegated to smaller print, and innovations were made, such as considering the functions of the parts being described.

As well as considering function, attention was also given to those other aspects of the fourth dimension —development and evolution. The embryology of organ-systems has for many years now been a standard part of the background of teaching gross human anatomy and, in most centres, at least some attention is also given to comparative anatomy, by way of emphasizing among other things the specializations involved in man having an upright posture. In some centres a more detailed study is made along almost zoological lines to paint in more of the history of the vertebrate body.

The beginnings of a serious study of the anatomy of domestic animals were made before the already highly developed human anatomy began to break with tradition. It was inevitable, therefore, that the method established in human anatomy was directly imitated. Having, however, to deal with some five different mammals, attempts at a comparative approach, although along purely descriptive lines, were made at the outset. As time passed, many teachers of veterinary anatomy grew aware of the deficiencies of the purely descriptive approach and introduced function and embryology along with a description of parts. But this was seldom a true grafting-on of new material. The current state of development of the teaching of human anatomy gives opportunities for radical changes in approach by veterinary anatomists along avenues less accessible to those that originated them.

Human anatomists are in a difficult situation in the matter of how much descriptive detail to include in their courses. Obviously, their students will need to know the structure of certain parts in considerable detail in their clinical years, and too much cannot be left to the surgeons to teach without a radical alteration being made in medical students' courses. Certain critics maintain that all descriptive anatomy should be applied anatomy and taught alongside surgery. Although this would obviate the difficulties inherent in expecting students to remember structural details with several years gap between pre-clinical anatomy and surgery, it would relegate anatomy to pure technological training. It has more to offer a student than that. But the problem of where to draw the descriptive line is certainly an acute one.

This particular problem is far less pressing in the teaching of veterinary pre-clinical anatomy, for the amount of applied descriptive detail required by even a specialist in surgery is far less. The detail chosen for treatment should, of course, be selected with one eye on its applied value; but even so, the main criterion of choice is its biological value. Which structural details can best be shown as structure-in-action? This is one of the basic yard-sticks for selection. Which details can best be treated as examples of adaptation to function against a background of evolutionary and developmental change? Finally, in this choice of what to leave out and what to put in—what other details must be included in order to give a rounded picture of the whole structuro-functional organism rather than merely a pastiche of disconnected parts.

Having, of necessity, to begin with a comparative approach, in order to deal with the structure of odd- and even-toed Ungulates and the Carnivora, among mammals, and of domestic poultry, among birds, it is obviously relevant to include something on reptiles, from which both later classes devolve. In turn, reptiles are only meaningful in matters of structure if their earliest representatives are coupled with early amphibians and treated as the first tetrapods in contrast to fish-like vertebrates. Thus, a more than superficial survey of the vertebrate body is a natural corollary to the teaching of veterinary anatomy. In this way it also joins forces with the dogfish, which the framers of early First M.B. courses in their wisdom included in its syllabus. In human anatomy courses the introduction of much comparative anatomy can be frowned upon as overloading the student. There is no need for a similar criticism in veterinary anatomy courses, where the subject-matter quite naturally demands it.

The embryology of the various organs of the mammalian body falls naturally into place alongside their evolutionary history and their gross form. That is to say, a unified course of all three approaches—not three separate courses. One of the major difficulties here is the absence of appropriate text-books. All the best text-books on mammalian anatomy and embryology are written for medical students. The various adaptations relating to upright posture and the rather more subtle differences due to precocity in development of many organs are not necessarily emphasized by the authors, for their subject-matter is man. For the veterinary student, these differences are a source of confusion, and time has to be consumed in interpreting them in terms of domestic animal anatomy. This very difficulty, however, contains an opportunity. Human anatomists give courses in quadrupedal anatomy. In veterinary anatomy there should be a course of comparative human anatomy. This would not only serve as a guide to existing text-books but would also fulfil two other wider and more valuable purposes—a means of liaison between medical and veterinary professions containing the elements of a common language between the two, and an important part of general education. Comparative medicine is now being given increasing attention; but the foundations of any training are laid in its early years—in this case, in pre-clinical courses. No opportunity should be lost in general education of increasing the spread of

knowledge about the human body, and for any student of animal biology a course on this subject should be a constant feature.

As well as having a long-established method of instruction and varied text-books for learning his anatomy, a medical student has another tremendous advantage over a veterinary student. He carries around with him a living text-book. To a non-medical observer, the behaviour of medical students while taking an oral examination is very interesting. All but the weaker candidates will make repeated reference to their own bodies when in the process of answering questions. Their examiners probably barely notice this, except to remark its absence in the poor candidates. It is a method of learning that comes naturally to the student, and all concerned, examiners and examinees, take it for granted. Although it has obvious restrictions, mainly to surface structures, it is nevertheless a means of knowing living anatomy that many a medical man almost certainly makes use of throughout his professional life. He adapts it later by asking patients to carry out various voluntary movements and noting the results, but his unconscious reference point is probably still his own body.

Both the approach and its adaptation would seem to be denied to a veterinary training. The use of living animals for palpation and observation, or the showing of films or cine-radiographs of living structures is never as effective, because full subjective participation on the part of the students is always absent. There is, however, a way around this difficulty. Fortunately, the most generalized of the domestic animals, the dog, differs in only a limited number of structural details from the anatomy of man. The comparative course in human anatomy could stress these differences to the veterinary student. By learning about his own body, which in itself contains an interest drive for any biology student, he can allow for the differences between his own anatomy and that of his subject animals and thereby use his own living text-book in a similar way to a medical student.

Modern anatomy is thus far from being a dead science, but, what of the criticism that it is too time-consuming in this age of specialization to warrant its continuance as a part of pre-clinical training, even in its modern forms? This criticism can perhaps be levelled even more readily at veterinary than at human anatomy because of the more restricted nature of its descriptive subject-matter. Most anatomists, after making full allowance for the axe they have to grind, are confident of the importance to the students of what they are seeking to do for them, in courses that must remain pre-clinical. The actual putting into words of what is often an instinctive conviction, accumulated through years of teaching, is however sometimes not easy to make. Surely one of the main answers lies in this matter of anatomy being a branch of biology? In this day and age where machines have invaded the world of biological research, and observation of living animals without interference experiments is a rarity, there is an increasing need to offset this mechanistic trend by laying foundations in the biology of the normal. The changes from the old descriptive method have rendered this possible, and, as technology advances, the importance of emphasizing what can now be taught as living anatomy will increase rather than diminish. By adding to the observations of living subjects the inside knowledge a student can have from his own body, use is made of the insight into a science which belongs to biology alone. Not to develop this insight in a world of advancing technology might well be to lose the very substance of that old but still undeniable claim that medicine, whether human or veterinary, is an art as well as a science.

[1] Woodger, J. H., "Biological Principles" (Kegan Paul, London, 1929).
[2] MacDougall, W., "The Riddle of Life" (Methuen, London, 1938).
[3] Russell, E. S., "The Behaviour of Animals" (Edward Arnold, London, 1938).
[4] Russell, E. S., "Form and Function" (John Murray, London, 1916).

RIBONUCLEIC ACIDS AND THE SYNTHESIS OF CELLULAR PROTEINS*

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IT is hard to believe nowadays how confused the whole field of nucleic acid biochemistry and localization was some thirty years ago: it was thought, for example, that thymonucleic acid was only found in animal cells, while yeast nucleic acid was a specific constituent of vegetal cells. We know now that thymonucleic acid is only one representative of the deoxyribonucleic acids, while yeast nucleic acid is just one of the many ribonucleic acids which are now known to exist in all kinds of cells. Regarding the role of deoxyribonucleic acid, all one could suggest was that it might play the part of an intranuclear *p*H buffer.

Progress and clarification in this field largely came from unexpected quarters, namely, from cytochemical and embryological work: for example, the introduction in the realm of cytochemistry of the specific Feulgen[1] reaction for deoxyribonucleic acid was a major event, since it led to the demonstration of two important facts: deoxyribonucleic acid is strictly localized in the chromatin of cell nuclei, and it is present in the nuclei of all cells, whether they are of animal or vegetal origin.

Work in the thirties by the Needhams[2] and by me[3] on the synthesis of nucleic acid in developing sea urchin eggs also led to important conclusions: it was found that cell multiplication, during embryonic development, is linked with the synthesis of deoxyribonucleic acid and that the site of this synthesis is strictly nuclear. No important reserve of deoxyribonucleic acid, sufficient to allow nuclear multiplication until the larval stages were reached, could be

* Substance of two Special University Lectures delivered at King's College, University of London, on January 26 and 27.

detected in the cytoplasm; the latter, however, already contains large amounts of nucleic acids, in unfertilized eggs. Since this nucleic acid cannot be deoxyribonucleic acid, it must be ribonucleic acid; the latter can therefore no longer be considered as a specific constituent of plant cells. The hypothesis of a conversion of ribo- into deoxyribonucleic acid during cell division was an apparently logical conclusion to draw from all this work. How much of these conclusions remains true nowadays? A good deal, since the universal occurrence of deoxyribonucleic acid in cell nuclei and its synthesis during cell division are now well-established facts; the presence of ribonucleic acid in the cytoplasm of all cells is also generally accepted. The only major flaw in the theory was the hypothesis of the conversion of ribo- into deoxyribo-nucleic acid, which was not on a sufficiently sound basis; but, although such conversion certainly does not occur on a very large scale during egg development, recent biochemical work has shown that many organisms, including embryos, contain enzymes which transform ribonucleosides into their deoxy-counterparts and, ultimately, into deoxyribonucleic acid.

More progress in our basic knowledge about the role and the localization of ribonucleic acid was to come from other cytochemical studies: a simple staining method, in which two basic dyes (methyl green and pyronine) are combined, allows quick and easy detection of ribonucleic acid in all cells; however, the specificity of the method must be checked by the utilization of control sections which have previously been treated with the enzyme ribonuclease in order to remove the ribonucleic acid they contained (Brachet[4]). Simultaneously, Caspersson[5] worked out a more complicated and more costly method of detection of ribonucleic acid based on the strong ultra-violet absorption of the purine and pyrimidine bases which enter into the constitution of the nucleic acids.

Extensive use of these two methods led, independently and simultaneously, Caspersson[5] and me[4] to the same general conclusion: ribonucleic acid is a universal constituent of all living cells, whether they are of animal, vegetal, or bacterial origin. It is localized in the nucleoli and in the cytoplasm, although variable amounts are found in chromatin as well. Furthermore, there is a remarkable correlation between the ribonucleic acid content of a given cell and its ability to carry out protein synthesis: cells which are metabolically very active, but do not synthesize large amounts of proteins (kidney, heart, muscle cells, for example) have a low ribonucleic acid content. On the other hand, cells which synthesize large quantities of enzymes in the pancreas or the gastric mucosa, as well as the silk-secreting cells of silk worms, contain very large amounts of ribonucleic acid in their cytoplasm and their nucleoli. Finally, when protein synthesis is increased or decreased by experimental means, such as fasting or hormone injection, parallel changes in the ribonucleic acid content of the affected cells can be detected.

More recently, the advent of a new and powerful cytochemical technique, autoradiography, has entirely confirmed these conclusions (Brachet and Ficq[6]): there is an excellent, almost perfect, parallelism between the incorporation of amino-acids into proteins (which is a measure of protein synthesis) and the ribonucleic acid content of all the cells which have been studied.

Quantitative Measurements

Since cytochemical methods do not lend themselves so easily to quantitative work as straight biochemical techniques, it was important to confirm the cytochemical results with quantitative methods: this has been done[7,8] repeatedly, and all that need be said here is that the agreement between the two types of technique is remarkable. We can thus from now on consider the correlation between the ribonucleic acid content and protein synthesis as a well-established fact, and we can draw the conclusion that ribonucleic acid somehow takes a direct role in protein synthesis.

A consequence of this conclusion is that there should also be a correlation between ribonucleic acid content and protein synthesis at the subcellular level: it means that the small particles rich in ribonucleic acid which have been known as microsomes since the work of Claude[9] should be more active in the incorporation of amino-acids into the proteins than the other cell fractions (nuclei, mitochondria, cell sap) which are poorer in ribonucleic acid. The correctness of this prediction has been confirmed many times and, more recently, it has been possible to go one step further and to isolate from the microsomes, by suitable treatment with bile salts, particles which are much smaller and have a still higher ribonucleic acid content than the microsomes: they are the so-called 'ribosomes', which contain as much as 40 per cent ribonucleic acid. It was found, as one would expect, that they are unusually active in the incorporation of labelled amino-acids into proteins[10]. In fact, these ribosomes are now considered by most biochemists as the main site of synthesis of cytoplasmic protein, in bacterial as well as in vegetal and animal cells. Of great interest also is the finding that treatment of microsomes by ribonuclease, in order to destroy the ribonucleic acid they contain, quickly inhibits their ability to synthesize proteins[11]. It can therefore be safely concluded that the above-mentioned relationship between ribonucleic acid content and protein synthesis holds true at the subcellular as well as the cellular or histological level; furthermore, the integrity of ribonucleic acid, in subcellular particles, is necessary if the ability to synthesize protein is to be retained.

But the first clear-cut demonstration of a direct role of ribonucleic acid in protein synthesis came from an entirely different field: the study of the multiplication of plant viruses, especially tobacco mosaic virus. After the demonstration by Markham[13] and by Jeener[12] that the protein part of tobacco mosaic virus (which consists of ribonucleic acid and protein only) is not infective, it was established beyond doubt by Gierer and Schramm[14] and by Fraenkel-Conrat[15] that viral ribonucleic acid, if properly extracted and isolated, is infectious. Treatment of this ribonucleic acid with ribonuclease immediately abolishes the infectivity. A very important point in these experiments is that when viral ribonucleic acid gets into tobacco leaves, it not only reproduces itself but also synthesizes its protein counterpart and produces virus of the strain from which it originates. In other words, ribonucleic acid is the genetic determinant in plant viruses (and in all animal viruses containing ribonucleic acid which have been studied so far) just as deoxyribonucleic acid is in phages and in cells.

But viruses are very simple systems when compared with whole, living cells; for the biologist, it would be

of the utmost interest to prove that the integrity of ribonucleic acid is required for protein synthesis in the case of living cells also. Experiments to test this possibility have been going on for several years in my laboratory; the technique is to add ribonuclease to the living cells and to follow such processes as growth, mitotic activity, incorporation of amino-acids into proteins and net protein synthesis. The main results can be summarized as follows[16]: ribonuclease easily penetrates many living cells, where it can accumulate to a large extent. It first breaks down the soluble (cell-sap) ribonucleic acid and then attacks microsomal and nucleolar ribonucleic acid by either breaking it down or building up an inactive enzyme–substrate complex. In any event, protein synthesis, growth and mitotic activity quickly stop, probably as a result of the initial breakdown of soluble ribonucleic acid. But it is a very remarkable fact that the deleterious effects of ribonuclease can be reversed to a large extent (and sometimes even completely) by the external addition of yeast ribonucleic acid. The experimenter can thus control, almost at will, growth and protein synthesis in living cells by playing upon the ribonucleic acid–ribonuclease equilibrium.

After these prolonged cytochemical and biological studies, the problem has finally reached the ultimate stage, the biochemical one: thanks to the work of Hoagland, Zamecnik and their colleagues[17], the mechanism of protein synthesis becomes more and more understandable. This synthesis involves three main stages: first, the amino-acids are activated, in the presence of adenosine triphosphate, by soluble enzymes, and this results in the formation of amino-acyl-adenosinemonophosphate compounds. Secondly, these compounds are incorporated into soluble ribonucleic acid; this step is very sensitive to ribonuclease in the *in vitro* biochemical experiments as well as in our own experiments with onion roots. Finally, the soluble ribonucleic acid, which now carries the amino-acid, is, in turn, incorporated into the ribosomes; there, by a mechanism which remains obscure in many respects, specific proteins, which possess the correct amino-acid sequence, are synthesized. There is no lack of hypotheses about this ultimate step of protein synthesis: the most probable one is that the ribosomal ribonucleic acid carries a 'code', which it has received from nuclear deoxyribonucleic acid; this code would be inscribed, in the ribonucleic acid molecule, in the form of a specific base sequence and it would be translated, in the finished protein, as a corresponding, specific amino-acid sequence.

Role of the Nucleus

Before we can accept such a control mechanism of cellular protein synthesis, we should know more about the role taken by the cell nucleus (which obviously contains all the genetic information) in the synthesis of ribonucleic acid and of protein.

Several methods are available for such investigations; although all of them have some serious drawbacks, it is very encouraging that they all lead to very similar conclusions. The three main methods which have been used so far are the following: (1) investigation of the incorporation of labelled precursors of nucleic acids or proteins into intact cells, by autoradiography methods; (2) comparison of nucleic acid and protein metabolism in nucleate and anucleate fragments of unicellular organisms or eggs; (3) work on homogenates, in which nuclei, mitochondria, microsomes and cell-sap constituents can be recovered by differential centrifugation. The main difficulties with these three types of methods are the following: with the autoradiography techniques, specific activities are often difficult to measure accurately, since usually we do not know exactly the intracellular concentration of the labelled precursor and the chemical composition of the cell organelle which is under study; in the case of anucleate and nucleate fragments of cells, the main limitation is that delicate ultramicromethods are necessary in order to make measurements; with homogenates, it is difficult to be sure that no adsorption or loss of chemical compounds has occurred as a result of a rather drastic procedure. Furthermore, results obtained with 'combined' homogenate fractions (nuclei + microsomes, for example) are always of doubtful significance, since the experimental conditions are too different from those which prevail in a living cell. After these preliminary, but necessary, remarks, I will now discuss briefly the results obtained with the three different types of approach.

Autoradiography work on intact cells has repeatedly emphasized the importance of the nucleolus and perinucleolar chromatin in ribonucleic acid metabolism; these nuclear constituents, in all the cases studied so far, become very quickly labelled when ribonucleic acid precursors such as adenine, orotic acid, cytidine, uridine, etc. are used. It looks as if these precursors could go through the whole of the cytoplasm without being used for the synthesis of ribonucleic acid but, once they reach the nucleus, they incorporate themselves almost immediately in the nucleolus and the surrounding chromatin. Later on, the cytoplasmic ribonucleic acid also becomes labelled to an appreciable extent. All the facts at hand thus suggest that synthesis of ribonucleic acid is extremely active in the nucleus and the adjoining heterochromatic segments; they also strongly suggest that ribonucleic acid which has been synthesized in the nucleus is transferred to the cytoplasm.

The situation is different in the case of protein synthesis: as a rule, nuclei do not incorporate labelled amino-acids at a much faster rate than the cytoplasm; the main exception of this rule is the case of eggs undergoing cleavage, where synthetic processes are practically restricted to the multiplication of the nuclei. These observations obviously suggest that the nucleus is more directly involved in ribonucleic acid than in protein synthesis (see Brachet[18] for a more complete discussion). Experiments recently carried out in my laboratory by Drs. R. Perry and M. Errera entirely confirm this general conclusion: destruction of the nucleolus by an ultra-violet microbeam exerts marked effects on the synthesis of ribonucleic acid in both cytoplasm and chromatin; but, on the other hand, it has relatively little effect on the synthesis of nuclear and cytoplasmic protein.

Nucleate and Anucleate Fragments

I now come to the work done on nucleate and anucleate fragments of unicellular organisms: in *Amoeba*, removal of the nucleus has little effect on the time of survival of the organism; but anucleate fragments quickly become unable to move and to feed: therefore they should be compared with their

nucleate counterparts under fasting conditions only. Under these conditions, it is found that removal of the nucleus does not decrease appreciably the oxygen consumption; however, the anucleate halves soon become incapable of utilizing their carbohydrate and fat reserves and, instead, proteins are being broken down[19]. This protein breakdown affects various specific (enzyme) proteins to different extents: whereas protease, adenosine triphosphatase, enolase and amylase remain almost unaffected by removal of the nucleus, dipeptidase undergoes a definite decrease, and acid phosphatase and esterase almost vanish from the anucleate halves. The reason why the nucleus exerts such markedly different effects on the maintenance of a number of enzymes remains uncertain; it may be connected with different intracellular localizations of the various enzymes which have been studied so far; but this point certainly requires additional experimental work before it can be cleared up. Finally, it should be added that the incorporation into proteins of labelled amino-acids, such as methionine or phenylalanine, is markedly reduced after enucleation; this incorporation process, which can be taken as an index of protein synthesis, never stops, however, in anucleate cytoplasm so long as the latter is alive.

The effects of removal of the nucleus are much more drastic, in *Amoeba*, in the case of ribonucleic acid than in that of proteins: the ribonucleic acid content of anucleate fragments decreases quickly and strongly[19]. Ingenious experiments by Goldstein and Plaut[20] have provided direct evidence for the view that the reason for this considerable drop in the ribonucleic acid content of anucleate cytoplasm is that a large proportion of cellular ribonucleic acid is synthesized in the nucleus: grafting the nucleus of an *Amoeba* which had been labelled with phosphorus-32 into a normal *Amoeba*, they found that cytoplasmic ribonucleic acid becomes labelled. On the other hand, the normal nucleus does not take any of the label into its ribonucleic acid, a fact which suggests that ribonucleic acid can move inside the cell in one direction only, namely, from the nucleus towards the cytoplasm.

In accordance with the results which have just been presented and which suggest that the nucleus is the main centre for the synthesis of ribonucleic acid in *Amoeba*, it has been found that enucleation exerts very impressive inhibitory effects on the incorporation of various precursors into cytoplasmic ribonucleic acid. Anucleate fragments seem, however, to be able to carry on, but at a much reduced rate, independent synthesis of cytoplasmic ribonucleic acid. But the whole situation still remains somewhat obscure, since the possibility that the incorporation process might occur in bacteria which have been phagocytized by the *Amoeba* without being killed has not yet been ruled out satisfactorily.

The biochemical activities of anucleate cytoplasm are much greater in the unicellular alga *Acetabularia mediterranea* than in *Amoeba*: it was found by Hämmerling[21] that anucleate halves not only survive for several months, but also that they are even capable of extensive morphogenesis (cap formation); it is therefore not surprising that these anucleate fragments are capable of net protein synthesis, including that of specific enzymes such as aldolase, phosphorylase and fructosidase[22–24]. Although the genes control, as we know, the synthesis of the many enzymes which are present in the cells, removal of the nucleus has nevertheless no immediate effect on enzyme synthesis. It should be said, however, that protein and enzyme synthesis cannot go on for ever in the absence of the nucleus, and that it stops completely after three or four weeks.

The case of *Acetabularia* raises a number of important problems for all those interested in molecular biology: morphogenesis and synthesis of specific proteins can obviously occur, in this case, in the complete absence of the genetic material. There must therefore be an intermediary between deoxyribonucleic acid and the proteins: Could it be that cytoplasmic ribonucleic acid carries the genetic information originating from the genes, and controls, for a certain time in any event, synthesis of specific protein? If so, is this ribonucleic acid of nuclear origin? Is it possible, by experimental means, to suppress the expression of the information which the anucleate cytoplasm has formerly received from the genes? While no clear-cut answer can be given to any of these important questions, recent experiments shed a certain amount of light on them.

Regarding first the behaviour of cytoplasmic ribonucleic acid in anucleate fragments, recent experiments by Dr. Naora and me have led to the following conclusions: there is, in contrast to what happens to protein, no net synthesis of ribonucleic acid in these fragments. On further analysis, however, it is found that a true synthesis of chloroplastic ribonucleic acid occurs in pieces of anucleate *Acetabularia*; this synthesis apparently takes place at the expense of the other cytoplasmic ribonucleic acid fractions (microsomes, cell-sap), which decrease after enucleation. We thus arrive at the following conclusions: chloroplasts show a high degree of independence towards the nucleus and can apparently multiply in its absence; microsomal and soluble ribonucleic acids, in *Acetabularia* as well as in *Amoeba*, lie under much closer nuclear control.

There is good reason for believing that, in *Acetabularia* as in *Amoeba*, part of the cytoplasmic ribonucleic acid is of nuclear origin: incorporation of ribonucleic acid precursors is extremely active in the nucleolus[18] and treatment of the anucleate halves with inhibitors of ribonucleic acid synthesis (ribonuclease, for example[25]) leads to an irreversible loss of regenerating capacity; a similar treatment of nucleate halves produces only a temporary inhibition of regeneration; these facts strongly suggest that the morphogenetic processes, in anucleate fragments, are mediated by ribonucleic acid of nuclear origin. It should, however, be emphasized that anucleate pieces incorporate radioactive precursors into their ribonucleic acid at an almost normal level for many weeks[22,23]: there is thus no doubt that, in this alga, pure cytoplasm is capable of extensive and independent synthesis or turnover of ribonucleic acid.

Regarding finally the expression of the gene-determined morphogenetic character (cap formation) in anucleate fragments, it is interesting to note that it can be easily controlled by modifying the SH—SS equilibrium of the culture medium[23]: by adding —SH-containing substances (β-mercaptoethanol for example), cap formation is completely suppressed. In the presence of its oxidized (—SS-containing) counterpart, dithiodiglycol, caps form earlier and develop faster than in the controls. In other words, the genetic information which the cytoplasm has received from the nucleus (through ribonucleic acid of nuclear origin presumably) is 'forgotten' in the presence of mercaptoethanol and expresses itself

better than normally when cytoplasmic —SH groups are oxidized.

Before we close the *Acetabularia* chapter, a last remark should be made: it would be a mistake to believe that nucleo-cytoplasmic interactions always correspond to nuclear control exerted on the cytoplasm; in this alga, a decrease in cytoplasmic energy production, by prolonged cultivation in the absence of light, for example, has far-reaching consequences on both the structure and the ribonucleic acid content of the nucleus and, especially, the nucleolus[16].

The experiments which have been performed on enucleate egg cells and on reticulocytes (see ref. 18 for a more complete review) are in substantial agreement with all that has been said about *Acetabularia*: removal of the nucleus in sea urchin or unfertilized eggs of the newt has no immediate effects on the incorporation of various precursors into ribonucleic acid and proteins; in reticulocytes, the loss of the nucleus is followed by a parallel drop in the ribonucleic acid content and the ability of the cell to incorporate amino-acids into proteins. Nevertheless, synthesis of various enzymes and even of hæmoglobin (the synthesis of which is very clearly genetically controlled) has been reported to occur in these anucleate cells: if this is true, it would be another piece of evidence for the view that genetically controlled synthesis of specific proteins can occur in the complete absence of the genes; here again, one could speculate that cytoplasmic ribonucleic acid of nuclear origin is the carrier of the genetic information.

Work on Homogenates

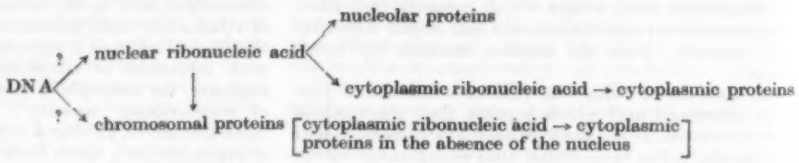
A few words should be said, finally, about the results obtained by biochemists who have been working on fractions recovered by differential centrifugation of homogenates (cf. ref. 18); it has been repeatedly shown, by this method, that the incorporation of ribonucleic acid precursors is always higher in nuclei than in any cytoplasmic fraction; similar experiments made with labelled amino-acids failed to demonstrate a particularly high ability of the nuclei for synthesizing proteins. Thus, we find once more that the nucleus is more directly involved in ribonucleic acid synthesis than in protein synthesis. Mention should be made, in this respect, of the very interesting experiments carried by Allfrey and Mirsky[17] on isolated thymus nuclei: the latter are capable of protein synthesis, and whole deoxyribonucleic acid is required in order to allow them to carry on this process. However, this role of deoxyribonucleic acid is an indirect one, since deoxyribonucleic acid is mainly concerned with energy production in the isolated nuclei. Furthermore, the experiments of Allfrey and Mirsky[17] clearly show that the ribonucleic acid synthesis is a necessary initial step before the isolated nuclei can incorporate amino-acids into their proteins.

General Conclusions

If we compare biological systems so different from each other as *Amoebae*, algae, eggs and reticulocytes, we find big quantitative differences; but they probably are just the result of a secondary factor, namely, the level of energy production in the anucleate cytoplasm. It is not surprising that an anucleate fragment of *Amoeba*, which is unable to feed, cannot be the site of a net protein synthesis; the situation is of course much better for *Acetabularia* (which is capable of active and normal photosynthesis in the absence of the nucleus), for eggs which are full of glycogen, fat and protein reserves, and for reticulocytes which live in such a rich nutrient medium as the blood stream.

Granting these easily understandable quantitative differences, there is no doubt that all the experiments made so far point toward the same general conclusion: the cell nucleus is the main site of synthesis of nucleic acids (deoxy- and ribonucleic acids). Its role in protein synthesis is a less direct one: it apparently controls the synthesis of cytoplasmic protein through the production of nuclear ribonucleic acid. The latter must, ultimately, receive the genetic information from deoxyribonucleic acid; but there is no evidence so far that 'deoxyribonucleic acid makes ribonucleic acid'. Nor is there any experimental evidence for the view that deoxyribonucleic acid could directly 'make' proteins, even chromosomal proteins. Finally, it should not be forgotten that, in the case of *Acetabularia*, independent synthesis of protein and ribonucleic acid (in the chloroplasts at least) is possible in the complete absence of the nucleus and, therefore, of genes and deoxyribonucleic acid.

For these reasons, the common 'slogan': DNA makes RNA, and RNA makes protein, is, in my opinion, an over-simplification which may prove dangerous. The following scheme, although still certainly very simple, is probably somewhat closer to the truth:



It will be a task for the future to remove the question marks and to increase the number of the arrows present in this still largely hypothetical scheme.

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THE AGADIR EARTHQUAKE OF FEBRUARY 29

By ERNEST TILLOTSON

THREE slight earth tremors during the previous week were not considered premonitory of the earthquake which suddenly occurred near Agadir at about 11.40 p.m. on the night of February 29, 1960, since this district had not previously experienced an earthquake of intensity 10 on the ten-degree scale. However, at 23h. 40m. 12s. U.T., after preliminary rumbling, the shock occurred. 90 per cent of the Talborjit quarter where many Moroccans lived in multi-storied apartment buildings, 70 per cent of the new buildings, including the Saada Hotel, built only five years ago, and the Gautier Hotel, and 20 per cent of the industrial buildings collapsed. Agadir, the tourist and economic capital of southern Morocco, where the Atlas Mountains descend to the Atlantic Ocean, was ruined. Some 12,000 people, including 3,000 foreigners, were estimated to have lost their lives, and many thousands besides were injured. A seismic sea wave (tsunami) which reached some 300 yards inland from the coast and then retreated, added to the damage.

There was no electric supply and there were no telephones; sporadic fires occurred as the fires in buildings were scattered about; the sanitary and water systems were utterly destroyed. The headquarters building for the militia collapsed with loss of life, but the police headquarters remained standing. Moreover, there were only a few police in the buildings during this night of Ramadan. An orphanage for about a hundred children was shattered; the hospital was badly damaged, and the maternity ward destroyed. Sailors from the French naval air base some 2½ miles away were quickly on the scene, and their prompt action saved thousands trapped under the ruins.

The ancient Casbah, inhabited by some 500 people, built about the year 1540 on high hills above the port, was destroyed. The shock was felt strongly in Mogador, about 100 miles to the north.

King Mohammed put his son, the Crown Prince Moulay Hassan, in charge of all necessary operations. Total evacuation of the population was ordered, the dead buried, and rescue operations for those trapped were continued. The injured were evacuated to hospitals in other parts of Morocco, and tented camps were erected, a large one being between Agadir and the Moorish town of Inezgane, 7 miles south-east of Agadir, which itself suffered some 30 per cent damage. Many buildings left unstable by the earthquake have been demolished and, since epidemics, especially of typhoid and plague, were feared, a 5-week quarantine was ordered. The ground has been spread where necessary with quicklime and 800 tons of DDT have been sprayed on the area.

At the port of Agadir all cranes collapsed with the falling-in of the quays and jetties, except on the main jetty. The principal cargo sheds were damaged, the harbourmaster killed and both pilots injured.

Evidence of a tremendous submarine upheaval off the coast was obtained from preliminary naval soundings. In one case the depth of water was found to be 45 ft. where previously it was charted at 1,200 ft. In a position about 9 miles from the shore, soundings showed a depth of 1,200 ft. instead of the charted 4,500 ft. An extensive hydrographic survey will thus be required, especially near the harbour, where some dredging was necessary even before the earthquake.

The shock was registered by seismographs at observatories throughout the world (Durham University Observatory, England, *iPz* 23h. 45m. 44s. *D*, *iSE* 23h. 50m. 21s. U.T.), and the United States Coast and Geodetic Survey placed the preliminary epicentre at lat. 30° N., long. 9° W. Small aftershocks have continued to occur, one at about 22·30h. on March 3, one about 00·01h. on March 4, two on March 5 and one on March 6.

The Times reported "an informed source" as stating that the insurance coverage in London for Agadir was expected to be a good deal short of one million pounds, "as North Africa is not regarded as a dangerous area for earthquakes" and as Agadir is basically under French influence and dealt with by French insurance companies. However, any rebuilding which may be undertaken at or near the present site of Agadir must now take earthquake risk into account. Earthquake damage at a given place depends on the properties of the elastic waves arriving there, on the ground at the site shaken, and on the structures involved. The ratio of amplitudes at sites on fairly dry alluvium more than 500 ft. deep to those at sites on crystalline rock is frequently 5 : 1, or more for earthquake waves of period 1–1½ sec. For waves of period about 0·1 sec. and for waves having periods exceeding 10 sec. (length more than 10 miles) the differences in amplitude are small. Amplitudes of earthquake waves at sites on water-saturated soft ground may be ten times those on crystalline rock. The period of waves giving the maximum ratio decreases to a quarter of a second for places with alluvium about 100 ft. thick. Moreover, at sites on alluvium the length of time of shaking also increases. Ground effects may produce these appreciable differences in duration and amplitude even at localities only a fraction of a mile apart. The importance of choosing crystalline rock or at least dry ground, and of avoiding water-saturated soft ground for foundations of buildings in order to reduce potential earthquake damage, cannot be over-emphasized.

OBITUARIES

Dr. W. F. P. McLintock, C.B.

William Francis Porter McLintock, formerly director of the Geological Survey of Great Britain, was born in Edinburgh on February 2, 1887, and was educated at George Heriot's School and the University of Edinburgh. He graduated in 1907, with special distinction in botany, in a remarkable science class of a dozen students which yielded two directors of Geological Surveys (Heron of India was the other), two principals and one professor in Dominion universities, and two heads of departments in the Indian Civil Service.

Following a Civil Service examination in mineralogy, McLintock was appointed assistant curator in the Museum of Practical Geology. Four years later, in 1911, he was promoted to take charge of the geological galleries of the Royal Scottish Museum; and when the Geological Survey and Museum was reorganized in 1920 under the directorship of (Sir) John S. Flett, he returned with Flett from Edinburgh to London, this time as curator. The Museum and Library at 28 Jermyn Street, built in 1851, had long been a reference centre for economic geologists and mining engineers, and a rapid growth of the collections there reflected McLintock's ever-helpful advice to the many that sought it.

In 1927, the Royal Commission on Government Museums recommended the transference of the Museum of Practical Geology to South Kensington, and under Flett's direction McLintock spent the following years in planning the new Museum. His clear-cut and revolutionary ideas on the need for a popularization of geology found small support in a tour of European institutions and met with little encouragement in some academic quarters at home; but a happy co-operation with H.M. Office of Works enabled him to achieve his ideals when the Geological Museum was opened to the public in 1935, at the centenary of the Geological Survey. Nothing in recent years has done more to promote the study of geology in Britain. From an attendance of 18,000 a year in Jermyn Street, the annual number of visitors soon soared to more than 250,000 and to-day exceeds 300,000.

When war came, the Museum was taken over as headquarters of the London Civil Defence Regional Organization, and the collections and library were in part dispersed to the country. McLintock, then deputy director of the Geological Survey, became responsible for the administration of the Survey's war effort, initiating departments dealing with raw materials for atomic energy, exploration geophysics, underground storage of petroleum, and other strategic fields. By 1945, when he succeeded as director, he had plans fully prepared to meet the heavy tasks of re-organizing the Survey on a much-expanded peace-time programme and rapidly restoring the Museum to public service. On completion of this work he resigned in October 1950.

McLintock's publications include a variety of papers on Cornish and Devon minerals, and on the zeolites of Mull; a model account of the Strathmore meteorite, the flight of which he himself observed from an Edinburgh street and which he acquired for the national collections the next morning: a guide to the Geological Museum's collection of gemstones, now in its third edition; and a petrographical study of unusual pseudo-igneous rocks collected by him in Persia, which were shown to be sediments fused in the combustion of oil and gas seepages long since extinguished. In a long and fruitful collaboration with Dr. J. Phemister, he organized and carried out pioneer gravitational and magnetic surveys at many localities in Britain, to demonstrate the usefulness of geophysical methods in geological exploration.

Many will recall the brilliance and pungency of his conversation, the precision of his long memory, the independence of his judgment, and the sharpness with which he devastated all that was hollow and unsound; but perhaps those colleagues who shared with him the long night watches in war-time London appreciated best his unswerving loyalty to the Geological Survey and unselfish devotion to the interests of its staff. He died suddenly in Edinburgh on February 21, following a happy decade of retirement, with his wife, in the Perthshire countryside.

C. F. Davidson

Prof. S. A. Mitchell

Prof. Samuel Alfred Mitchell, formerly director of the Leander McCormick Observatory, died in his eighty-sixth year at Bloomington, Indiana, on February 22. Born at Kingston, Ontario, he graduated at Queen's University, Kingston, in 1894, took a degree at Johns Hopkins University and became instructor and professor of astronomy at Columbia University. In 1912, he was appointed director of the Leander McCormick Observatory, Virginia, a post that he held until his retirement in 1945.

His chief work there was the determination of stellar parallaxes by photographic methods. He was a member of the Commission on Stellar Parallaxes of the International Astronomical Union from its foundation and followed Schlesinger as chairman of the Commission in 1928. He was also a member of the Commissions on Variable Stars, Solar Physics and Solar Eclipses. He was chairman of the last-named Commission from 1935 until 1950 and remained a member of it until 1958.

It is for his work on eclipses and on his speciality throughout, the chromospheric spectrum, that Mitchell will be best remembered. He went on ten eclipse expeditions—four in the United States and one each in Sumatra, Spain, Norway, Niauafoou (or Tin-can Island), Canton Island and Canada; in only three cases were the eclipse observations entirely spoilt by clouds—California in 1923, Norway in 1927 and Canada in 1932. Probably he saw the corona on more occasions than any one else. His book "Eclipses of the Sun" gives an account of his experiences.

Mitchell received the Watson Gold Medal of the U.S. National Academy of Sciences and was elected an associate of the Royal Astronomical Society in 1933, shortly after he had become qualified by naturalization as a citizen of the United States.

Thereto hangs an amusing story. When he married he was still a Canadian, and according to the law of the United States at that time his wife became a Canadian. Later he became naturalized as an American citizen, but the law had meanwhile been altered and the wife's nationality no longer followed her husband's; Mrs. Mitchell remained a Canadian under United States law but became American under British law. Later, wishing to visit her son, who was studying in England, she had to be naturalized as an American citizen in order to get a passport to come to England!

F. J. M. STRATTON

Dr. George A. Edwards

GEORGE A. EDWARDS, associate research scientist in micromorphology, at the Division of Laboratories and Research, New York State Department of Health, died in Albany on March 1, at the age of forty-six. Dr. Edwards was a graduate of Tufts University 1939, and received his Ph.D. in biology from Harvard University, 1947. Prior to going to the New York State Department of Health in 1956, Dr. Edwards had been professor of general physiology, co-ordinator of research, and co-founder of the Marine Biological Laboratory, University of São Paulo, Brazil, from 1952; he was a corresponding member of the Brazilian Academy of Sciences.

In the Second World War, Dr. Edwards was aviation physiologist attached to the Air Force in the development of equipment for high-altitude flying and survival techniques for air-sea rescue. Dr. Edwards did extensive research on nerve and muscle physiology, pharmacology, and morphology. His recent publications in electron microscopy included also studies of the ultra-structure of several dimorphic pathogenic fungi and the micromorphology of human bronchogenic carcinomas.

NEWS and VIEWS

Physics at Liverpool: Prof. J. M. Cassels, F.R.S.

PROF. J. M. CASSELS has been appointed to the Lyon Jones chair of physics at the University of Liverpool in succession to Prof. H. W. B. Skinner, who died in January. Prof. Cassels was educated at Rochester House School, Edinburgh, St. Lawrence College, Ramsgate, and Trinity College, Cambridge. Although best known for his many contributions to high-energy physics, his earliest work was performed at the opposite extreme of the energy-scale in nuclear physics when he carried out an elegant and painstaking research in the scattering of very slow neutrons by solids, using the Cavendish Laboratory cyclotron in collaboration with R. Latham. His introduction to high-energy physics was at the Atomic Energy Research Establishment, Harwell, where he worked with the 175-MeV. protons of the synchrocyclotron between 1949 and 1953, producing many papers on nucleon-nucleon scattering and high-energy nuclear interactions. From there he moved to the bigger synchrocyclotron at Liverpool, first as a lecturer and in 1956 as professor of experimental physics. His main research interests were now in pion physics, and he built up a strong group working in this field. He was elected to the Royal Society at the early age of thirty-four. In 1959 he took an appointment at Cornell University with R. R. Wilson, where he has been using the 1·2-GeV. electron synchrotron in electron scattering experiments. He was closely associated with CERN during its earliest days, and has maintained close contacts with high-energy physics at the Atomic Energy Research Establishment and the Rutherford Laboratory.

Metallurgy at Leeds: Prof. J. Nutting

PROF. J. NUTTING, who succeeds Prof. N. J. Petch (see *Nature*, **183**, 1643; 1959) in the chair of metallurgy in the University of Leeds, was educated at Mirfield Grammar School, Yorkshire (1935–42), and then at the Department of Metallurgy, University of Leeds (1942–45), where he was awarded the degree of B.Sc. with first-class honours in metallurgy. During the period 1945–48 he worked under the late Prof. Preece on the overheating and burning of steel and was awarded the degree of Ph.D. in 1948. He then went to Cambridge and worked for one year in the Cavendish Laboratory as a scientific officer for the British Iron and Steel Research Association and there joined with Dr. V. E. Cosslett and Prof. E. Orowan on the development of techniques for applying the electron microscope to metallurgical problems. In 1949 he became a demonstrator, and in 1954 a University lecturer, in the Department of Metallurgy, University of Cambridge. For the past twelve years he has worked on problems of phase changes in both ferrous and non-ferrous alloys and on the relation between phase changes and mechanical properties of alloys. He has been particularly interested in electron microscopy and its application to these problems of alloys. He has travelled and lectured widely at scientific conferences and in 1951 was awarded a Nuffield travelling fellowship to visit Canada and the United States.

A New British Computer

ON April 6 there was demonstrated in London the first commercially built British digital differential analyser. This machine, the *Corsair*, has been produced as the result of the joint activities of the Royal Aircraft Establishment, Farnborough, and Semiconductors, Ltd. The differential analyser as such is an old device, but the use of digital circuits to perform the operation of integration has infused it with new life and accuracy. The main attraction of a differential analyser, considered as an analogue device, is that it performs continuous operations such as integration at a much higher rate than is possible with a programmed digital computer. Analogue computing elements are, however, inaccurate, and it is difficult to attain precisions of more than about 1 per cent. The digital differential analyser overcomes this trouble by reducing the integration to digital summation, because this can be performed simply and at very high speed. In digital circuits accuracy is no problem, so that the *Corsair* is able to attain an accuracy of better than 0·01 per cent, which quite outclasses purely analogue devices.

The new computer uses about 2,000 transistors of which nearly 700 are of the surface barrier type, the characteristics of which permit the direct current

connexion of various elements with consequent saving of complexity and space. The machine has the rather large number of 50 integrators, which gives it great power and versatility, its physical size is quite small—about 20 c. ft.—and its appearance is attractive and neat. When it is produced in quantity, it should prove an invaluable research tool in universities, colleges of technology and in industry. It is pleasant to see that Britain has now entered this important new field.

Imperial Chemical Industries, Ltd.

A new ammonia plant, with a capacity of 100,000 tons a year, and associated plants to make urea and fertilizers is to be built at Imperial Chemical Industries, Ltd., Severnside Works in south Gloucestershire, at a cost of upwards of £10 millions. This plant is expected to start production during 1963. This is the second important project that Imperial Chemical Industries has decided to construct on the new 1,000-acre Severnside site. The first, announced in February, will involve the construction of plants to make ethylene oxide and ethylene glycol, costing £5 millions. Imperial Chemical Industries has also decided to extend the methanol plant at its Heysham Works in Lancashire. A new plant to make 30,000 tons a year began production there only a few months ago. A further extension to make an additional 45,000 tons a year is now to be built, and is expected to be completed during 1962. Methanol is an important raw material for the plastics industry.

Scientific and Technical Services in Rhodesia and Nyasaland

A list has recently been published of government and statutory bodies concerned with scientific research and technical services in the Federation of Rhodesia and Nyasaland, giving in brief the history, functions, organization, location and establishment of each (Government Organizations and Statutory Bodies concerned with Scientific Research and Technical Services in the Federation of Rhodesia and Nyasaland, 1959. Pp. 80. Salisbury: Office of the Federal Prime Minister and External Affairs, 1959). Research within the Federation is treated as a "concurrent subject", that is to say, that both the Federal and Territorial Governments have power to legislate in this field. The Federal Government obtained the services of Mr. D. G. Kingwell, of the South African Council for Scientific and Industrial Research, to report on the development of science in the Federation. Mr. Kingwell's recommendations have been adopted, and a Research Act has been brought into operation which is designed to promote scientific and industrial research, including agricultural, veterinary and tsetse research, to establish a research foundation and to provide for the establishment of research councils.

Organization of Medical Care

A report by a World Health Organization Committee on medical care embodies the opinion of a group of experts from several countries, representing public health administration, hospital administration, medical practice, health insurance and voluntary agencies, on measures for "the organization and co-ordination of comprehensive preventive and curative medical care services provided to the individual outside the hospital wards" (World Health Organization. Technical Report Series No. 176: Role of Hospitals in Ambulatory and Domiciliary Medical Care—Second Report of the Expert Committee on Organization of Medical Care. Pp. 32. (Geneva: World Health Organization; London: H.M. Stationery Office, 1959.) 1 Swiss franc; 1s. 9d.; 0·30 dollar.) The scope of such services is considered to cover ambulatory care in out-patient departments, domiciliary care, after-care and medical rehabilitation. While recognizing that the degree of co-ordination possible will depend on national or local conditions, the Committee considers that the establishment of a central authority with planning and advisory powers would facilitate the rational siting of services and maintain the standards of both the care given and the equipment available. The main out-patient services which would be expected to contribute to a programme of ambulatory and domiciliary medical care are defined, and their functions indicated. They are: dispensaries, polyclinics, out-patient departments, health centres, medical practitioners, industrial medical services, medical rehabilitation centres, and intermediate institutions such as day-hospitals and night hostels. It is emphasized that continuity of care is of the utmost importance; this involves provision for an easy transition from domiciliary and ambulatory to hospital care, and back to the physician first concerned with the case.

The National Research Development Corporation of India

The fifth annual report and statement of accounts of the National Research Development Corporation of India covers the period ending March 31, 1959, and records sustained progress (pp. 23. New Delhi: National Research Development Corporation of India, 1959). Commercial production from several patents and processes licensed in previous years has already commenced and twenty-four licences were negotiated compared with seventeen in the previous year, and five processes went into production. Development expenditure on projects already sanctioned amounted to Rs. 3.91 lakhs, and gross income from premiums and royalties increased to Rs. 1.66 lakhs. Marketing of 'Hykol' brand activated carbons produced at the Regional Research Laboratory, Hyderabad, commenced, and mica insulating bricks and carbons produced by Corporation licencees have been used in the Bhilai steel plant. During the year 56 inventions were reported for development by 20 research institutions, bringing the total of reported inventions to 503. Projects for the refining of cotton seed oil and the production of copper chlorophyll from indigenous raw materials were completed, and progress is reported in the projects for phthalic anhydride, de-ionization of cane juice, white cement from feldspar, and the fluidized-bed technique for textile processing.

Inventory of Economic Studies in Africa

The Commission for Technical Co-operation in Africa South of the Sahara has issued an annotated reading list of books, articles and official publications since 1945 dealing with economics in Africa (CSA/CCTA Publication No. 30; Joint Project No. 4: Inventory of Economic Studies concerning Africa South of the Sahara—an Annotated Reading List of Books, Articles and Official Publications. Pp. xi+301. (London: Commission for Technical Co-operation in Africa South of the Sahara, 1960)). The inventory, which has been edited by Miss Peter Ady on the basis of information collected by a panel of national correspondents in economics, is the out-

come of the adoption by the Commission in 1957 of the recommendation of a Specialist Committee in Economics. Its 1,377 entries are confined almost entirely to publications dealing specifically with African regions or countries, but it has proved unusually difficult to draw the line between economics and anthropology and between political economy and politics. The entries are arranged alphabetically by author, first, geographically, under Africa General; Belgian Congo; French Republic and Community; Portuguese Africa; Spanish Africa; British Africa; Ghana, Central Africa; and South Africa. Within these sections the entries are classified by subject and there is an author index. Entries and annotations are in English and French.

Museum and Art Gallery, Durban

THE annual report of the Durban Museum and Art Gallery for the municipal year 1958–59 records that curatorial work has mainly centred on the new Marine Hall (pp. 19. Durban: Durban Museum and Art Gallery, 1959). The exhibits of sharks, rays, skates, turtle and Cape sealion have been installed and work on a large exhibit of the major game fishes of the Natal coast is nearly complete. After many experiments plastic has been used to good effect in these recent marine displays. The output of research work on the collections by the staff remains at a high level. Display and storage accommodation at the Old House Museum (which serves as a branch Folk Museum) is now so limited that serious consideration must be given in the relatively near future to the provision of an additional building, either as a separate entity in the grounds or as a wing of the Old House.

Field Studies Council

MEASURED by the quality of the courses conducted, the number of students attending the centres, and the financial outcome of the year's operation, the report of the Field Studies Council for 1958–59 is again one of continued progress. (Pp. 34+4 plates. London: Field Studies Council, 1960.) Over the past nine years the total number of bookings at the Centres has risen from 3,483 to 7,903. Of these, more than half were students of biological subjects, and more than a quarter of geographical and geological subjects. A surprising number of those attending took courses in art and archæology. Well over half the students attending were from grammar schools but, regrettably, the numbers attending from secondary modern schools remained very small; this is a matter to which education authorities should be asked to pay closer attention. Because of the establishment of a new centre at Slapton Ley, the Ministry of Education generously agreed not to carry out its declared intention progressively to reduce the £5,000 annual grant. The reduction will now begin next year and will not lead to much embarrassment. Further information concerning the work of the Council can be obtained from Mrs. F. H. C. Butler, Ravensmead, Keston, Kent.

Reference Books on Mineralogy in the U.S.S.R.

THE Academy of Sciences of the U.S.S.R. is planning to publish in 1959–65 a collection of reference books on mineralogy. The subjects covered by the projected volumes are as follows: Vol. 1, introduction, native elements, intermetallic compounds, arsenides and antimonides, sulphides; Vol. 2, haloids; Vol. 3, silicates (part 1); Vol. 4, silicates (part 2); Vol. 5, niobates and tantalates, carbonates, nitrates, borates, sulphates; Vol. 6, phosphates, arsenates and vanadates, tungstates, molybdates, etc.; Vol. 7, diagrams illustrating phase equilibria important in mineralogy. The size of each volume is estimated to be 500 pages and the price 38–40 roubles. This collection of reference books will be prepared under the auspices of the Institute of the Geology of Ore Deposits, Petrography, Mineralogy and Geochemistry, and of the Mineralogical Museum of the Academy of Sciences.

Scientific Bibliography of Latin America

THE Conference of Latin American Scientific Experts convened by the United Nations Educational, Scientific and Cultural Organization at Montevideo in September 1948 requested the preparation of a Latin American biobibliography (Unesco: Centro de Cooperacion Cientifica para America Latina. Scientific Institutions and Scientists in Latin-America. Paraguay (1er Volumen). Pp. ii+93. Chile (2° Volumen). Pp. ii+40. Montevideo: Unesco, Centro de Cooperacion Cientifica para America Latina, 1959). The preparation of this was undertaken by Unesco's Field Science Co-operation Office for Latin America in collaboration with Ibero-American organizations, and Vol. 1 contains a first list of Paraguayan scientists and scientific organizations. Scientists are listed alphabetically, with particulars of their qualifications and experience and publications. Vol. 2 gives similar data for institutions and scientists in Chile.

Ionosphere over Belgium during the International Geophysical Year

THE Royal Meteorological Institute of Belgium has been publishing monthly bulletins of the ionospheric observations taken at the station of Dourbres (lat. 50° 06′ N., long. 04° 36′ E.) during the International Geophysical Year (Royal Meteorological Institute of Belgium, Avenue Circulaire, 3, Uccle, Brussels, 18). An automatic ionosonde of the U.S. National Bureau of Standards type *C–2* was used; this sweeps over the range of frequencies from 1 to 25 Mc./s. in 30 sec., emitting 30 pulses per sec. of 50 μsec. duration. The peak power transmitted is about 10 kW., from one of two vertical rhombic aerials used for the lower and upper parts of this frequency range (1–4 and 4–25 Mc./sec.). On normal days, soundings are made at about fifteen-minute intervals; but during the Regular World Days and Special World Intervals of the International Geophysical Year, soundings were made every five minutes.

The bulletins of Ionospheric Observations taken during July–October 1958, inclusive, have recently been issued. These are similar in form to those issued for the preceding months of the International Geophysical Year; and contain graphs of the median monthly values of the heights and critical frequencies of the *E*, *F*1 and *F*2 regions, and also curves showing the percentage of the total time during which the critical frequency of the sporadic *Es* layer exceeded the limiting values of 3, 5 and 7 Mc./sec. In addition to these graphs, the individual hourly observed values are given for the *F*2, *F*1, *E* and *Es* layers. Additional tables give the maximum usable frequencies for radio transmission over a distance of 3,000 km. by the *F*2 and *F*1 layers, and other values relating to the height and critical frequency of the sporadic *E* region. The symbols used throughout the bulletins conform with the decisions of the Union Radio-Scientifique Internationale/International Geophysical Year sub-committee on Ionospheric Soundings.

Modern Explosives

Number 5 of the Royal Institute of Chemistry "Lectures, Monographs and Reports, 1959" covers the subject of modern explosives (by the late Dr. Wilfrid Taylor. Pp. 63. London: Royal Institute of Chemistry, 1959. 6*s*. net). It is a well-balanced review of modern chemical explosives, written by one of the most distinguished research workers on the subject who, until his death in 1958, was for many years in the Research Department of the Nobel Division of Imperial Chemical Industries, Ltd. It is an excellent introduction for the chemist, physicist or engineer, to the scientific basis of the development of chemical explosives. Dr. Taylor describes in succession the early history of the development of high explosives, the properties sought from explosives, the chemical composition of the more important individual explosives and of the various commercial mixtures developed from them to suit various requirements. He follows with sections on the physical phenomena of detonation (velocity of detonation, temperatures and pressures developed during detonation), and on the precise physical action of explosives in breaking rock and coal. Next is a brief but useful statement of the hydrodynamical theory of detonation, followed by a discussion of modern views of reaction mechanisms in detonation. A special section is devoted to coal-mining explosives, which have to reach the highest possible safety against the danger of ignition of firedamp and coal dust. The whole subject is condensed into sixty very readable pages, with a couple of pages of references carefully selected from among the thousands of original papers on the subject.

A Radiocarbon Dating System using Scintillation Techniques

A procedure for radiocarbon dating, based on the use of scintillation techniques, is described by C. F. G. Delaney and I. R. McAulay (*Sci. Proc. Roy. Dublin Soc.*, A, 1, No. 1; 1959. 4*s*.). The method involves the conversion of the sample into methyl alcohol, which is then mixed with a liquid scintillator and its radiocarbon activity measured, in a quartz cell, using an arrangement of two photomultipliers in coincidence. The overall yield in the chemical preparation is about 45 per cent, and the purity of the methyl alcohol is checked by gas-phase chromatography. The background counting-rate is reduced by the use of massive screening combined with the application of the technique of pulse-height selection to the outputs of the photomultipliers. In this way, a background of 13·6 c.p.m. is obtained, and the net counting-rate for contemporary material is 20·6 c.p.m. above this value. Thus, when using a counting period of 17 hr., results of significance to 30,000 years are obtainable. The weight of sample required (equivalent to about 15 gm. carbon) is rather large compared with the requirements of most of the methods in current use, nearly all of which are based on proportional gas-counting techniques. However, this method, unlike those based in gas counting, has the advantage that it is not dependent on supplies of liquid air (which is not readily available in Dublin). Moreover, the preparative chemistry is relatively simple, and less steel screening is required than is necessary in most arrangements using proportional counters. The initial dating programme was concerned with the dating of vegetational changes detected by pollen analysis in a peat column from Redbog, Co. Louth, and the results of these measurements are presented.

X-Ray Studies of Some Carbonized Coals

In a recent paper, R. Diamond has described a notable advance in the X-ray study of carbonaceous materials (*Phil. Trans. Roy. Soc.*, 252, 193; 1959). The paper reviews the author's detailed work with P. B. Hirsch on the X-ray examination of a range of coals carbonized up to 1,000° C., and brings together the analysis of variations in aromatic layer size distribution, mean C—C bond-length and molecular packing in a consistent model of the carbonization process. The approach to the problem of aromatic layer size distribution is particularly interesting not only because it is the first attempt of this kind, but also because of the elegance of the method. The author uses matrix methods in fitting a linear combination of theoretical intensity functions for graphitic layers of various size-groups to the observed X-ray intensities with the minimum total square error. The results are given as histograms showing the percentages by weight of material associated with each size-group. It would seem that this 'resolution' is approaching the limit attainable by X-ray analysis of these complex disordered materials. This is brought out in Diamond's careful discussion of the significance of layer diameter measurements when the structural model is modified to include edge groupings and departures of the aromatic layers from a truly graphitic structure. The structural chemist will want to know more about the nature of the aromatic systems as well as other features which X-ray analysis cannot elucidate, but the facts brought to light in this work should appreciably limit the range for conjecture in a field where it has inevitably been wide.

Culture of Salmon and Trout

In many parts of North America, the culture of trout and salmon is carried out on a large scale, and a bulletin recently issued by the Department of Fish and Game for the State of California deals briefly with many aspects of hatchery practice (No. 107: Trout and Salmon Culture—(Hatchery Methods). By Earl Leitritz. Pp. 169. Sacramento: Printing Division, Department of Fish and Game, 1959. 2 dollars). The sections on feeding practices and the treatment of disease serve to illustrate how new methods and techniques have been introduced during the past ten or fifteen years. Comprehensive feeding schedules have been developed from actual production records, and in this way the fish are adequately fed with a minimum of waste and the minimum amount of labour. Various anæsthetics have been employed as an aid in tagging, taking of spawn and the transportation of fish. Until recently, urethane (ethyl carbonate) was perhaps the most common anæsthetic used in fishery work. However, tests with mammals have shown that this substance could be carcinogenic after prolonged and repeated exposures. Because of this risk to fishery workers, urethane is no longer used as a fish anæsthetic in California. As an alternative, *MS* 222 (tricaine methane sulphonate) is in common use both in the United States and Europe.

This book was prepared at the request of many of the Department's fish hatchery personnel, and naturally it is concerned mainly with the culture of rainbow trout and certain species of Pacific salmon. However, it contains much useful data and practical

information, and would serve as a handy reference for anyone concerned with the rearing and care of salmonids in Britain.

Late Precambrian Fauna in South Australia

In 1947, R. C. Sprigg first described fossilized soft-bodied animals in what were then believed to be lower Cambrian quartzites near the Ediacara mining area, some 380 road miles north of Adelaide, South Australia. In view of its scientific importance, the area was proclaimed a fossil reserve in May 1958, under the control of the Minister of Education and the authorities of the South Australian Museum. In October 1958, M. F. Glaessner and B. Daily visited the area; they have now described its geology and remarkable fauna (*Rec. South Austral. Mus.*, **13**; July 1959). It is estimated that eight hundred fossil specimens have been collected from near the top of the Pound Quartzite at Ediacara. Work on this material is not complete; but it is now thought that the fauna consists not only of medusæ believed to represent the Scyphozoa and Hydrozoa, but also Anthozoa (Octacorallia), Annelida, and at least two entirely new types of invertebrates. One or more elements of this fauna show relations with the fauna of the Nama System of South Africa, and one with *Charnia masoni* Ford from Charnwood Forest, England. Glaessner suggests that stratigraphic and palæontological evidence supports the placing of this fauna in the Late Precambrian rather than the Lower Cambrian. The fauna is marine, with both pelagic and benthonic elements, and the lithology suggests shallow water. All the forms appear to have been soft-bodied, and have been preserved as moulds and casts, almost entirely on the undersides of the flaggy quartzite beds.

Evidence of Concurrent Translocation

The problems of translocation in plants remain enigmatic and of perennial interest. In a recent investigation, evidence that different kinds of translocation take place concurrently has been obtained by C. D. Nelson, H. J. Perkins and P. R. Gorham (*Canadian J. Bot.*, **37**, No. 6, 1181; 1959). The method used was to trace the pathway of photosynthetically assimilated carbon-14 in the soya bean. They found that when the primary leaf of a soya bean plant was allowed to carry on photosynthesis in labelled carbon dioxide, a small amount of labelled carbon was translocated downward into the root at a velocity of at least 5,040 cm. per hour. This velocity was fifty times greater than the velocity at which sucrose was translocated in the phloem. The rapidly translocated carbon-14 was not moved as carbon dioxide. When the time of translocation was increased from 30 sec. to 10 min. there was an increase in carbon-14 content of the root without any increase in carbon-14 content of the lowest part of the stem. Part of the carbon-14 was translocated through a steam-girdled stem, but under these conditions there was no accumulation in the root. This indicates that the rapid translocation of carbon-14 occurred in both living and dead tissues of the stem. These results were correlated with the localization of carbon-14 in tissue autoradiographs of cross-sections of the stem to show that there were at least three pathways of downward translocation: (*a*) a slow translocation in the phloem; (*b*) a rapid translocation in the xylem; and (*c*) a rapid translocation in either the cortex, the phloem, or the pith.

Grooved Rocks at Apoje, Nigeria

A report of some investigations on grooved rocks at Apoje, near Ijebu-Igbo, Western Nigeria, by William Fagg appeared in the December issue of *Man*. Only a few rocks were studied, but it would appear that there are many more in the region. The grooves, which are deep, wide and rounded, cannot be said to be anything in the way of figures, though circles and ellipses do occur. The problem naturally arises as to the age of these carvings and who made them. They do not recall other rock carvings described by H. Fosbrooke in *Man* during 1952, where it was shown that they were connected with initiation ceremonies. At the same time, these new finds are too carefully made to be merely the result of grinding and polishing stone tools. The modern inhabitants hold them in reverence, and this would suggest a certain antiquity and the probability that they were made by folk who preceded the ancestors of the present population. Maybe when other examples are discovered and studied the answer to these queries will become plain.

Australopithecine Tools

Bone tools at the Kalkbank Middle Stone Age site and the Matapansgat Australopithecine locality, Central Transvaal, are the subject of an article by Prof. R. A. Dart, Dr. Kitching and Revil J. Mason (*Arch. Bull.*, **13**, No. 51; Sept. 1958). For some time, Prof. Dart has been studying what kind of bones very early man selected for possible tool-making purposes. Such a selective process would indicate some degree of intelligence. If it can be shown that even the Australopithecine used bone for simple tool-making, and selected certain kinds of bones and perhaps preferred certain animals, then a little more can be suggested as regards the intelligence of these lowly creatures.

Nematology at the Imperial College of Science and Technology

A new Nematology Laboratory for the Imperial College of Science and Technology, London, to be built and equipped at the College Field Station at Silwood Park, near Ascot, Berkshire, at a cost of £30,000, will be the first university laboratory in the Commonwealth specifically staffed and equipped for research and training in this subject at postgraduate level. Shell International Chemical Co. is contributing £15,000 towards the cost of building and equipping the Laboratory which, it is hoped, will be completed in time for the new academic year beginning next October. Under Prof. B. G. Peters, who was appointed to a new chair of parasitology in Imperial College in 1955 (see *Nature*, **176**, 145; 1955), work on nematology has been developing, and a full-time one-year course in nematology was started in 1958.

Fritz London Award for Low-Temperature Research

Nominations for the second Fritz London International Award are being solicited from all low-temperature scientists by the selection committee for this award. The first award was made to Dr. Nicholas Kurti, of the Clarendon Laboratory, Oxford, at the fifth International Conference on Low-temperature Physics and Chemistry in Madison, Wisconsin, during August 1957. If a suitable candidate is found the award will be made at the seventh International

Conference on Low-temperature Physics to be held in Toronto during August 29–September 3. Further information can be obtained from Dr. F. G. Brickwedde, College of Chemistry and Physics, Pennsylvania State University, Pennsylvania.

The German Geological Association: Jubilee Meeting

The Geologische Vereinigung held its jubilee meeting at Würzburg during March 12–14 and it was attended by more than four hundred geologists. The theme considered was orogens. Some thirty authors contributed, their papers being grouped under the headings: time and matter in orogenesis, and structure and distribution of orogens. In the first part the concept of geosynclines was discussed and the sedimentation, metamorphism and the part played by magma within them enlarged upon. In the second, special structures within mountain chains of five continents were described and the differences between those near, or on, the surface and those existing at depth were contrasted. The principal award of the Association, the Steinmann Medal, was made to Prof. H. H. Read, emeritus professor of geology in the University of London, for his outstanding contributions to geological science.

Helminthological Society of Washington Anniversary

The Helminthological Society of Washington will observe its fiftieth anniversary on October 8. The scientific programme will be held in the University of Maryland, College Park, Maryland. A banquet will be held during the evening at the Naval Officers Club at Bethesda, Maryland. Dr. Chauncey D. Leake, president of the American Association for the Advancement of Science, will be the speaker at a banquet to be held on that day.

Wool Textile Research Conference

The second Quinquennial Wool Textile Conference is to be held in the United Kingdom during May 18–28. The conference, the theme of which is "Fibre Science", is to be arranged by the U.K. Textile Institute and is to be held at Harrogate. Participants from other countries include: Australia, Belgium, Canada, Germany, Japan, Netherlands, New Zealand, Norway, South Africa, Sweden, Switzerland and the United States. Further details can be obtained from the Textile Institute, 10 Blackfriars Street, Manchester 3.

Summer Meeting of the Illuminating Engineering Society

The Illuminating Engineering Society is holding its biennial summer meeting at Harrogate during May 15–18. Three papers will be presented: "Progress in the Field of Electric Lamps", by H. G. Jenkins (General Electric Co., Ltd.); "Rationally Recommended Illumination Levels", by H. C. Weston; "Plastics, Yesterday, To-day and Tomorrow", by C. L. Child (Imperial Chemical Industries, Ltd.). Further information can be obtained from the Secretary, Illuminating Engineering Society, 32 Victoria Street, London, S.W.1.

Announcements

H.M. The Queen, who is Patron of the Royal Society, will open the tercentenary celebrations of the Society at a meeting in the Royal Albert Hall on the afternoon of July 19 (see *Nature*, March 26, p. 879).

Dr. D. H. Follett, keeper of the Department of Electrical Engineering and Communications in the Science Museum, South Kensington, has been appointed director of the Museum in succession to Dr. T. C. S. Morrison-Scott, who is succeeding Sir Gavin de Beer at the British Museum (Natural History) (see *Nature*, March 26, p. 888). Mr. D. Chilton has been appointed keeper of the Department of Electrical Engineering and Communications.

Mr. R. C. Palmer, deputy director of research to Griffin and George, Ltd., and associated companies, has been appointed director of research in succession to Dr. A. J. P. Martin, who wishes to concentrate in future primarily on fundamental research. Mr. Palmer was on the staff of the Wool Industries Research Association, Leeds, during 1937–51, after which he was appointed director of research of the South African Wool Industrial Research Institute.

Mr. Lawrence Sutton, formerly director of studies at Ashridge College, has been appointed education officer to the Institution of Works Managers, 196 Shaftesbury Avenue, London, W.C.2. As such, he will collaborate with technical colleges running the Institution's two-year training course leading to the certificate in works management, of which more than a hundred were awarded in 1959.

Applications are invited for the Bowles Memorial Travel Scholarship. The object of the Scholarship is to enable the holder to travel abroad for the purpose of either collecting plants for introduction to British gardens or studying practical horticulture in other countries. Applicants must be of the male sex, not more than twenty-eight years of age on January 1, 1961, and have some academic connexion with horticulture. Applications should reach the Secretary, Royal Horticultural Society, Vincent Square, London, S.W.1, by September 30.

Under the title "Postgraduate Courses 1960–61" the Imperial College of Science and Technology has issued a booklet giving particulars of postgraduate courses available leading to a degree or diploma and of shorter postgraduate courses (mainly lectures) for which, under appropriate conditions, certificates are also given (pp. xii + 113. London: Imperial College of Science and Technology (University of London), 1960). Many of the courses have been accepted by the Department of Scientific and Industrial Research as suitable for the tenure of Advanced Course Studentships, and grants for the courses in agricultural chemistry and nematology are awarded by the Ministry of Agriculture, Fisheries and Food.

The seventh General Assembly of the International Union for Conservation of Nature and Natural Resources will be held during June 15–24 in Warsaw. The Assembly is to be held jointly with the eighth Technical Meeting; the final sessions will take place in Cracow. Further information can be obtained from the Secretariat of the International Union for Conservation of Nature, 31 rue Vautier, Brussels.

Erratum. In the communication entitled "Methods for the Evaluation of the Antibacterial Activity of Surface-Active Compounds" in *Nature* of April 9, p. 132, col. 2, line 47, for "fats" read "fate". The line now reads "The fate of microorganisms in dishwater was investigated . . ."

THE CARNEGIE INSTITUTION OF WASHINGTON

THE report of the Carnegie Institution of Washington, covering the year ended June 30, 1959, includes the report of the President, those of Departments and Special Studies, to which bibliographies are appended, and the administrative reports*. In reviewing the work of the year, the President directs special attention to the work of the Geophysical Laboratory and the Department of Terrestrial Magnetism and particularly that concerned with the mechanism of formation, the age and composition of Earth minerals. Using special equipment designed to reproduce conditions of temperature and pressure occurring within the Earth's crust, studies of mineralogical changes at pressures up to 50,000 bars (equivalent to a depth of about 150 km.) have shown that orthopyroxene (a calcium–magnesium–iron silicate with manganese) and minerals rich in alumina common in crustal rocks will react under conditions of temperature and pressure found in the mantle to form the magnesian garnet, pyrope. Experimental petrological examinations have been made of pyroxenes, garnets, biotites, amphiboles, spinels and feldspars and investigations of nickel–sulphur binary, iron–arsenic–sulphur and nickel–arsenic–sulphur ternary systems completed. The theory of 'coloured space groups' within crystals was extended and the application of the extended theory to seven mineral compounds of the cubic type of crystal structure was studied. Chemical studies of the highly refractory substance kerogen have been directed at its mode of formation and structure, and pilot experiments indicated that glucose and glycine could be transformed to substances suggesting asphaltine or even kerogen, but of different solubility, by incubating mixtures of them at 100° C. and *p*H values within the range which might have obtained in sedimentary rocks at the time of kerogen formation.

The techniques of mineral dating by nuclear clocks have been further extended and the ages of two or more co-existing phases in single rocks determined. Work on rocks in the Appalachian complex indicates that major geological events separated by very long intervening periods may be recorded by different minerals in the same rock. Further evidence has been obtained on questions of mountain building and it is now planned to make use of local earthquakes for crustal studies. The first station of a local network for detecting seismic disturbances planned for Peru, Bolivia and Chile was installed near Arequipa during the year. These stations will be equipped with electronically operated seismic recorders requiring the minimum of attention. A study of the mechanisms of heat and electrical conduction of the Earth and their relations concentrated during the year on the photon energy absorption of ferromagnesium silicates showed that the strong ultra-violet absorption of iron-bearing olivines was correlated with the presence of iron in the minerals .

* Carnegie Institution of Washington Year Book No. 58, July 1, 1958–June 30, 1959. Pp. xl+500+15 plates. (Washington, D.C.: Carnegie Institution of Washington, 1959.) 1 dollar, paper bound; 1.50 dollars, cloth bound.

In preliminary studies of all the non-thermal or 'noise-storm' events occurring during the past two years on the Sun no correlation has so far been found between the 'noise-storms' and optical features. The solar observational programme was extensively revised on January 1, 1959, and many older types of observations were discontinued or drastically curtailed to allow systematic examination of the newly discovered weaker magnetic fields which cover much of the solar surface. Observations of Mars with the 200-in. telescope in October 1958 by a guest investigator, Dr. W. N. Sinton, established the presence of three distinct absorption bands near 3·5 microns, chiefly in the dark areas and only very weakly in the bright areas, and in approximately the same positions as are found the reflexion spectra of terrestrial plants, and providing the most suggestive evidence yet to appear for the hypothesis that the dark areas on Mars are caused by some form of plant life which develops seasonally in these areas. Four new supernovæ were discovered during the year, two in nearby galaxies being bright enough for detailed observation. The chemical compositions of a large number of stars have been studied, and the observations and those on colour–magnitude relations are being correlated with data on the generation of nuclear energy and energy transfer from star core to star surface, and it is beginning to appear that the magnitudes and other properties of stars of a given class are affected substantially by the large differences in chemical composition observed. The Committee on Image Tubes for Telescopes has reported thirty-fold reduction in exposure time with two types of image tube, one a single-stage tube, including a photocathode, provisions for electron optics, and a phosphor screen coated on mica, and the other composed of two simple one-stage image tubes cascaded in series, with the phosphor screen of the second stage being photographed, using a fast-relay lens; but both types still have shortcomings.

In the Department of Terrestrial Magnetism the Biophysics Section continues to devote much attention to the study of ribonucleoprotein particles found in bacteria, and striking progress was made during the year in applying quantitative methods to such problems. Improved methods were developed for separating ribosomes of different sedimentation constants by centrifuging through a sucrose density gradient, and by incorporating radioactive tracers in cells for very short periods it was possible to observe nascent protein which is associated with bacterial ribosomes for a short period before it is released as a finished product. Bacterial ribosomes had the same function in protein synthesis as the ribosomes of higher organisms, but the speed of synthesis is about sixty times greater. The new measurements also confirmed the earlier hypothesis as to the nature of ribosome particles and the intricacy of the synthesis of ribosomes. The nucleotide compositions of several classes of ribonucleic acid from *Escherichia coli* were determined by isotope-

dilution techniques, using chromatographic and electrophoretic separations, and further information was obtained on the enzyme content of ribosomes, while the mechanism of protein synthesis was also studied through the amino-acid analogue technique, and considerable information was obtained on the mechanism of amino-acid selection by cells.

In the Department of Plant Biology it has been demonstrated that simultaneous absorption of light by chlorophyll *a* and chlorophyll *b* both increases the efficiency of light utilization by chlorophyll *a* and raises the initial photosynthetic rate after a dark period markedly over a situation when chlorophyll *a* alone is activated by light. Enhancement effects were also observed from alternate pulsing, in periods of 0·6–15 sec., of light sources of wave-lengths absorbed by chlorophyll *a* on one hand and chlorophyll *b* on the other. Studies continued on the characterization of the different forms of chlorophyll *a* occurring in living plants and additional evidence was sought for the differentiation of the three forms of chlorophyll *a* through fluorescence spectrography, differential bleaching and in other ways. By high-speed centrifugation in a liquid medium of graded density, protochlorophyll holochrome has been obtained relatively free from blue and ultra-violet absorbing contaminants and the transformation from protochlorophyll to chlorophyll was studied by observing the degree of fluorescence of freshly forming chlorophyll. Electron-microscopical studies of etiolated plastids showed that chloroplast lamellæ will form in the absence of protochlorophyll, but the normal aggregation and close pairing of the lamellar disks require the presence of chlorophyll. A bacterial pigment with protochlorophyll-like absorption properties was extracted from a mutant of the purple bacterium, *Rhodopseudomonas spheroides*. The gathering of essential data for the study of range grass hybrids of *Poa* is being concluded, and the investigations have revealed principles relating to the synthesis of new forms in apomictic genera by interspecific hybridization. A study on the genetic structure and evolution of the yarrows, members of the polyploid *Achillea millefolium* complex, is also nearing completion, and studies on rates of photosynthesis and respiration of two altitudinal races of *Mimulus cardinalis*, one from sea-level near the coast of California and the other at middle altitudes in the Sierra Nevada of California, have shown them to differ in the temperature optima and requirements for light saturation when functioning as intact plants. A device has been installed that can regulate, within a narrow range, the carbon dioxide in the air of a small chamber, thus permitting study of the effects of different concentrations of carbon dioxide on plants and simplifying the control of air temperature and relative humidity. In an analysis of the chromosome constitution of races of maize grown in Chile, Bolivia, Ecuador and Venezuela, a relation was found between the knob pattern and the geographical location of the race, which suggests that, under some conditions, a knowledge of the patterns of distribution based on chromosomal-knob constitution may give valuable evidence of the origins and migrations and past history of some early races of Indian corn.

The Department of Genetics has continued to focus attention on the structural and functional properties of genes and chromosomes, and it now appears that the suppressor–mutator system of control of gene expression in maize has some major resemblances to the system controlling flagellar–antigen formation in *Salmonella*. Studies of the effects of deoxyribonuclease on unfixed cells have shown that this enzyme enters the living cell and there brings about marked swelling and deformation of chromosomes, sometimes without appreciable loss of deoxyribonucleic acid. Hybrids have been obtained of *Salmonella typhimurium* and *E. coli*, thus permitting comparison of the homology of their chromosome structure, and the chromosome of the bacteriophage *T2* has been shown to consist of deoxyribonucleic acid, while the mechanisms of replication and recombination of genetic material in bacteriophages has also been investigated.

Studies of the synthesis and interaction of specific macromolecules, in the Department of Embryology, have been specially concerned with tissue specificity, and further experiments in immuno-embryology suggest that there may be two stages in the development of the immune response, which may be experimentally separable. Continued study of immune response of new-born rabbits to injections of chicken liver and spleen homogenates suggests that animals not injected at birth respond strongly to a few major antigenic components, whereas the response of the treated experimental group is eliminated or reduced. A study of the effects of thyroxine upon the metamorphosis of visual pigment of the eyes of the bullfrog tadpole afforded substantial support for the view that thyroxine acts directly on a rate-limiting factor in the eye itself. Encouraging results have been obtained in experiments on the inductive ability of tissue microsomes designed to elucidate inconsistencies in embryonic induction, attributed to a failure of ribonucleic acid or ribonucleoprotein to reach its site of action.

STATUS AND DEVELOPMENT OF THE TEACHING OF CHEMISTRY

At the Organization for European Economic Co-operation's Summer School for Science Teachers held in 1958 at Tutzing, Germany, the opinion was expressed that chemistry teaching in secondary schools has changed little since 1900 and that the present descriptive approach to the subject involves much memory work on the part of the student, and displays little of the scientific method. It was agreed that it was urgent that chemistry should be taught from the perspective of present-day theory and that so far as possible modern knowledge should be fed back to the school. As a result of these discussions the Organization for European Economic Co-operation's Office for Scientific and Technical Personnel, which works under the direction of Dr. Alexander King, in co-operation with the Irish Department of Education, organized a seminar at Greystones, Co. Wicklow, during February 28–March 10.

The objective of the seminar was to consider at a high level the preconditions and possibilities of: (1) a revision of chemistry teaching based on the knowledge of the atom; (2) pruning of the subject-matter in order to give more time to fundamentals without, however, neglecting adequate experimental work; (3) teaching chemical principles in such a way that they play a part in training the intellect of the pupil. The seminar was asked to come to conclusions concerning the specific steps required to provide the basis for a curriculum more fitted, intellectually and practically, to the needs of modern chemistry.

The following countries were represented at the seminar: Austria, Belgium, Denmark, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, Turkey, United Kingdom and United States. The participants comprised senior secondary school teachers of chemistry, those with official responsibility for chemistry programmes (senior officials of education ministries, inspectors of education), representatives of teacher-training institutes, professors of chemistry.

The proceedings were officially opened by Mr. Rafferty, secretary of the Irish Department of Education. Then followed the 'Keystone Address' of the chairman (Prof. T. S. Wheeler, Dublin). He recapitulated the objectives of the seminar and reviewed the papers to be presented, which were as follows: "Advances in Theoretical Chemistry since 1900" (Prof. K. Grob, Zurich); "The Importance of Molecular Structure in Elementary Chemical Teaching" (Prof. J. Bénard, Paris); "The Requirements for Entry to University Departments of Chemistry" (Prof. W. Cocker, Dublin); "The Requirements for School-leavers entering the Chemical Industry" (Dr. A. K. Mills, Dublin); "The Principles underlying Recent Advances in Practical Chemistry" (Dr. R. J. Magee, Belfast); "The Co-ordination of the Teaching of Chemistry, Physics and Mathematics in Secondary Schools" (Prof. M. Eurin, Paris); "The Teaching of Oxidation-Reduction and Acid-Base Reactions in Relation to the Electronic Atom" (Prof. A. Steiger, Zurich); "The Teaching of Chemistry to Students of 14–16 Years" (Dr. K. Stein, Bad Segeberg); "The Teaching of Chemistry to Students of 16–18 Years" (Dr. B. Borén, Stockholm); "Pruning and Simplification of the Syllabus and the Teaching of Fundamentals" (Prof. L. E. Strong, Richmond, Indiana); "New Materials and Teaching Aids, Text-books, Films, Models" (Prof. J. A. Campbell, Claremont, California).

The discussions were not confined to the set sessions, but were vigorous throughout the period of the seminar. Tuesday and the morning of Wednesday of the second week were devoted to the preparation of reports for transmission to the Organization for European Economic Co-operation. Owing to language difficulties, it was necessary to constitute three working parties (English and German; English and French; English or French, simultaneous interpretation). These parties were asked to make recommendations relating to two chemistry courses, one for students of 14–16 years and the other for those of 16–18. It was assumed that some three hours a week would be available for the study of the subject and that there would be facilities for experimental work.

It was realized that among countries conditions differ much as regards school-leaving age, facilities for practical work, time available for chemistry, and qualifications of teachers. In addition, schools have to cater for those who hope to become chemists and for others who have done with their chemistry when they leave school. Nevertheless it was considered that reports of the type envisaged would be of value to teachers anxious to modernize their courses, who could select to suit their needs.

Each working party had two chairmen (morning and afternoon) who kept in touch with the chairmen of other groups to ensure a measure of unity in the three approaches to the problems under consideration, though it was not thought disadvantageous to have three sets of views on the subjects at issue.

The reports were discussed at two plenary sessions of the seminar. It was agreed that the junior student should have his interest developed early in the constitution and properties of matter. Emphasis should be on observation, and formal definitions kept to a minimum. Methods other than the inductive arguments of Dalton should be used to develop the concept of atoms. The importance of space-filling models at all stages of instruction was stressed. The combustion of coke, the interaction of sulphur and copper, and similar systems can be employed to introduce the notions of chemical reactions, energy change, gaseous, liquid, and plastic systems, chemical equations and structural ideas. It is important that the student understand weight relationships and their correlation to chemical formulæ and equations.

For senior students atomic structure should be the framework of the course and not simply an additional chapter unco-ordinated with the rest of the chemical material presented. The Bohr–Rutherford atom provides a useful introduction; the nature of bonds should be discussed and directional characteristics emphasized. The law of mass action and chemical equilibrium should be treated in terms of reaction kinetics. The Brønsted treatment of acid-base equilibrium is recommended; redox systems can usefully be considered. Reaction mechanisms, thermochemistry and electrochemistry should also be treated in an advanced school course. So far as possible, phenomena should be related to structure; consideration of ion size can be illuminating in the study of crystals. The concept of electronegativity can also be employed to advantage in the discussion of chemical reactions.

Organic chemists should also be taught from the electronic point of view. The nature of single, double and triple bonds should be explained and the reactions of functional groups displayed in relation to their structure. The use of experimental results in the assignment of organic structures provides excellent examples of the application of the scientific method. If time permits, stereoisomerism should be discussed.

In all school courses the collection of a great number of facts about elements and compounds should be avoided. There is no necessity for systematic treatment of elements; examples should be drawn from the chemistry of one or more elements to illustrate a subject, for example redox systems, under discussion. Brief descriptions of technical processes should be introduced where they are of value in illustrating chemical principles. In this connexion reference was made to the tendency for accounts of out-of-date plant to be retained in school text-books. Properly prepared films and film strips can be of great help at all levels of instruction.

The seminar stressed the necessity for providing teaching of physics and mathematics such that the chemistry student has at all stages a knowledge of these ancillary subjects adequate for his needs. Finally, it was suggested that the Organization for European Economic Co-operation bring the recommendations to the notice of the appropriate authorities in the member countries with a request for comments. After an appropriate time small working parties, constituted similarly to the seminar, should meet in Paris to consider such comments as are received and to prepare manuals outlining school courses in chemistry relating to : (*a*) lectures ; (*b*) demonstration experiments by teachers ; (*c*) laboratory work by students. The necessity for devising means for training teachers capable of dealing with these new courses should also be considered.

The participants in the seminar felt that their discussion about a new approach to chemistry had been worth while, and that many concrete ideas of practical value had been elaborated.

T. S. Wheeler

WATER POLLUTION BY TRADE EFFLUENTS

THE last two major Acts in Britain governing the disposal of sewage and industrial effluents were the Public Health (Drainage of Trade Premises) Act of 1937 and the Rivers (Prevention of Pollution) Act of 1951 ; River Boards, which are responsible for controlling the discharge of polluting liquors to rivers, were set up by an Act passed in 1948. During the past few years, some of the provisions of the Acts of 1937 and 1951 have led to much discussion ; a committee, under the chairmanship of Sir Frederick Armer, set up by the Central Advisory Water Committee, after hearing evidence from the many interests concerned with pollution of surface waters, has now reviewed the experience of the past twenty years, and has recommended some important changes in the law*.

The Rivers (Prevention of Pollution) Act differentiated between discharges to streams already being made in 1951, and discharges made after that date. A River Board could control existing effluents in either of two ways : it could bring an action against anyone discharging a "poisonous, noxious, or polluting" liquid, it being left to the Court to decide whether the liquid could be so described, or it could make by-laws (which would require confirmation) covering a stream, or part of a stream, specifying standards of quality with which discharges would have to conform. It had been widely hoped and expected that River Boards would formulate by-laws, at least in industrial areas, since a manufacturer, for example, in designing a plant to treat a polluting liquor, clearly wishes to know the specification of the final effluent he is called on to produce.

It has been found, however, to be very difficult to formulate by-laws, and in fact none has so far been confirmed and come into operation. Since any standards adopted were expected to apply to a considerable length of river, if not to the whole of a river system, the first difficulty was to draw up a specification of permitted effluents which might have to be applied to a wide range of industrial wastes, the composition of which might be unknown, even to the manufacturers, and which might be so complex in composition as to make routine chemical analysis impracticable ; an example would be a liquor arising from the cooling and washing of a town's gas supply. This perhaps could have been overcome by examining liquors of whatever kind by a few comparatively simple tests, each designed to measure the effect of the liquor on some property of a stream. Among those suggested were tests for *p*H value, toxicity to fish, and concentration of suspended solids and oxidizable matter. A greater difficulty is that it is impracticable to treat all polluting liquors, particularly industrial liquors, to give effluents of a common standard of quality—a point which, from internal evidence, was clearly in the minds of those who drew up the Act of 1951.

Control of new or altered discharges brought into use after 1951 was, however, effected in a different way. Here a River Board could impose conditions on the nature and composition, and on the volume, of the discharges, each being considered separately. It was thus possible in each case to draw up a specification appropriate to the liquid to be discharged ; for example, for a liquor from electroplating, the maximum allowable concentrations of toxic metals and of cyanide would be specified. This machinery has worked very well, and since 1951 agreement has been reached on the conditions of discharge of large numbers of new effluents.

The Committee now recommends that River Boards should be empowered (subject to appeal) to deal with all discharges, whether new or existing, by essentially the same procedure. It was recognized that the improvement of the quality of some rivers, particularly the more heavily polluted of them in industrial areas, will have to be a gradual process. It is proposed, therefore, that a River Board should have powers to impose standards on effluents which could be reviewed later, and, if necessary, made more stringent.

On the question of discharge of industrial wastes to sewers of a local authority, the chief change recommended is that, except where a contract is in force, the authority should now be empowered to make a charge for the treatment of liquors which have been discharged without major change since before 1937 ; under the Act of 1937 these acquired 'prescriptive rights' and were exempt from payment. All traders would have to furnish information to their local authorities on the volume and nature of effluents discharged to sewers.

The Committee took evidence from all the major interests concerned with the purity of streams and, on all but one matter (the private rights of a riparian owner) have produced a unanimous report, which if implemented by legislation should greatly assist River Boards and local authorities in reducing the level of pollution of rivers in Great Britain.

* Ministry of Housing and Local Government : Central Advisory Water Committee. Final Report of the Trade Effluents Sub-Committee of the Central Advisory Water Committee. Pp. iv + 52. (London : H.M. Stationery Office, 1960.) 3*s*. 6*d*. net.

IRIDIUM–PLATINUM ALLOYS

DR. A. S. DARLING has recently published a critical discussion of the constitution and properties of the iridium–platinum alloys*. Until comparatively recently it was generally believed that these metals were completely miscible in each other in both the liquid and solid states—the liquidus curve, lattice parameters, electrical resistivity and microstructures all suggesting a continuous series of solid solutions. At the same time, there were other facts difficult to reconcile with such a view. As early as 1886 Le Chatelier had observed a thermal abnormality in the 20 per cent iridium alloy around 700° C., and in 1940 Masing, Eckhardt and Kloiber settled the matter by showing that a natural crystal from the Urals had a well-defined two-phase structure.

The true nature of the equilibrium diagram was finally established by Raub and Plate (*Z. Metallkunde*, **47**, 688; 1956), who showed that there is a miscibility gap with a maximum at 975° C. and 50 atomic per cent. At 700° C. this gap extends from 7 to 99 atomic per cent of iridium. That earlier workers had failed to appreciate the true state of affairs was due to the extreme sluggishness of the change, annealing periods of the order of a year being required to establish "some semblance of equilibrium" in parts of the two-phase region. The most accurate method of determining this phase-boundary appears to be from measurements of electrical resistance.

The strength and hardness of the alloys increase rapidly with the iridium content, 30 per cent raising the tensile strength from 10 up to 56 tons per sq. in. for the alloy quenched from 1,100° C., the Vickers Pyramid Number increasing seven-fold. Cold-working has an equally marked effect, and the tensile strength of cold-drawn 20 per cent iridium wire may exceed 100 tons per sq. in. Very high hardness values can, as would be expected from the diagram, also be produced by quenching followed by subsequent ageing, the 50 per cent alloy quenched from 1,400° C. and reheated to 700° C. for 15 hr. having a Vickers Pyramid Number around 500. It is not clear, however, whether the fully aged alloys retain sufficient ductility to permit the hardness to be raised still further by cold work.

Alloys containing up to 40 per cent of iridium have been hot-forged and even drawn into wire, and when melted in the argon arc, alloys of still higher iridium contents can be worked with difficulty. The presence of iridium considerably increases the resistance of platinum to corrosion, the 40 per cent alloy being virtually unattacked by boiling aqua regia. The high evaporation losses of these alloys at high temperatures is attributed to the volatility of iridium oxide, a matter of importance in connexion with their use in pyrometry.

Among the more important uses of these alloys Dr. Darling mentions their applications in electrical contacts, precision resistors and potentiometer slide-wires. The 10 per cent iridium alloy used in combination with a 40 per cent palladium–gold wire which generates an electromotive force of 34 millivolts at 600° C. forms a useful thermocouple for moderate temperatures. Bridge wires in electric detonators, fuses and cartridge igniters, the needles used for the initial perforation of diamond wire-drawing dies, suspensions for scientific instruments and hair-springs are other uses to which these alloys have been put.

* *Platinum Metals Rev.*, **4**, No. 1; Jan. 1960.

DEVELOPMENT OF LEARNING IN THE RHESUS MONKEY

DURING the past five years Harry F. Harlow and his colleagues have conducted an integrated series of researches into the learning capabilities of rhesus monkeys from birth to intellectual maturity*. Control over the monkey's environment has been achieved by separating the infants from their mothers at birth and raising them independently.

Half a decade is too brief a period to establish a definitive programme on the maturation of learning ability in the rhesus monkey, particularly in the later age-ranges. Within this period of time, however, techniques and tests have been developed which demonstrate that such a programme is feasible. The monkey is capable of solving simple learning problems during the first few days of life, and its capability of solving ever-increasingly complex problems matures progressively, probably for four to five years.

Early in life, new learning abilities appear rather suddenly within the space of a few days, but, from late infancy onward, the appearance of new learning powers is characterized by developmental stages during which particular performances progressively improve. There is a time at which increasingly difficult problems can first be solved, and a considerable delayed period before they can be solved with full adult efficiency.

The monkey possesses learning capacities far in excess of those of any infra-human primate, abilities comparable to those of low-level human imbeciles. The monkey's learning capabilities give little or no information concerning human language, and only incomplete information relating to thinking.

These are the generalizable limits of learning research on rhesus monkeys, but they provide an animal with vast research potentialities. There is a wealth of learning problems which the monkey can master, and at present the field is incompletely explored. The maturation of any learning function can be traced, and the nature and mechanisms underlying interproblem and intertask transfer can be assessed. Great research potentialities exist in analysing the fundamental similarities and differences among simple and complex learnings within a single species. The monkey is a subject ideally suited for studies involving neurological, biochemical and pharmacological correlates of behaviour. Rhesus monkeys can be raised under completely controlled conditions throughout a large part and, probably, all of their life-span, and the research of the future will probably correlate the neurophysiological variables, not with the behaviour of the static monkey, but with the behaviour of the monkey in terms of ontogenetic development.

* *American Scientist*, **47**, No. 4; December 1959.

SCIENTIFIC RESEARCH IN ANTARCTICA

THE Antarctic Treaty signed at Washington on December 1, 1959, by the British Government and by twelve others, all with interests, scientific or otherwise, in the Antarctic, including all seven claimants to the Antarctic's territories, recognized, in a preamble, that it is in the interests of all mankind that Antarctica should continue to be used exclusively for peaceful purposes and should not become the scene or object of international discord. The Treaty also recognized the extreme importance of scientific work and the firm foundation for the continuation of that work resulting from the International Geophysical Year.

In a short debate on the Antarctic in the House of Lords on February 18, opened by Lord Shackleton, who asked when the Government expected the Treaty to be ratified and what its policy was with regard to the future Antarctica, Lord Shackleton stressed the great value of the Antarctic as a source of scientific information. While that scientific interest had led to a remarkable degree of international co-operation, Lord Shackleton was somewhat concerned as to the possibility and effect of development, geologically and in other ways. In particular, he asked what provision had been made for the continuing discussion contemplated under Article 9 of the Treaty and what type of administration the Government had in mind. The Bishop of Norwich, who asked for a Government assurance that the British contribution in Antarctic investigation and research under the Treaty would not be less than that at present existing, also stressed the value of Antarctic research in long-range weather forecasting, geophysics, glaciology and some branches of biology; and after a tribute to the work done by Great Britain in the past, he emphasized the value of the opportunity for international collaboration in scientific work which the Antarctic offered.

Lord Lansdowne, who replied for the Government, said that it hoped to ratify the Treaty at an early date and that the Government's policy was to promote the basic aims of the Treaty. The Treaty should ensure the use of Antarctica for peaceful purposes only and it was hoped that it had established a firm foundation for scientific co-operation. The Government would have preferred a more comprehensive system of administration, but this could not be achieved at the conference. The question of some form of permanent secretariat was now under discussion. On the exchange of strictly scientific information and the planning of research, which came under Article 3, the Government considered that governments should not attempt to supersede the existing organizations such as the Special Committee on Antarctic Research, which had been working effectively since 1958. This Article, in fact, said: "every encouragement shall be given to the establishment of co-operative working relations with those Specialized Agencies of the United Nations and other international organizations having a scientific or technical interest in Antarctica". He hoped we would continue to make full use of such recently created organization and of the Specialized Agencies of the United Nations. The Government expected the Falkland Islands Dependency Survey, which was a principal contributor to Antarctic research, to continue, and those people most immediately affected by the Treaty, such as observers, exchange scientists and their staffs were, in fact, covered by the juridical and administrative arrangements.

VISUAL PIGMENT OF THE HORSESHOE CRAB, *LIMULUS POLYPHEMUS*

By Dr RUTH HUBBARD and Prof. GEORGE WALD

Biological Laboratories, Harvard University, Cambridge, Mass.

OWING mainly to the work of Hartline, the eye of the horseshoe crab, *Limulus*, has long been a classic object for electrophysiological investigation. This was the first eye in which electrical responses were recorded from single visual receptors[1]. The large size of these units and the relative simplicity of their nervous connexions have permitted a degree of analysis not achieved with other types of visual element[2]. This growing body of electrophysiological information lends special interest to the chemistry of the underlying processes.

We have now extracted the visual pigment of *Limulus*. This is the fourth arthropod visual pigment to be examined and the first such pigment obtained from an arachnoid. Two visual pigments have been extracted earlier from crustaceans, one from a euphausiid[3], the other from a decapod, the lobster[4]; and a visual pigment has also been obtained from an insect, the honey-bee[5].

The principal results of our experiments are summarized in Fig. 1. From a fraction of the eye tissues which we believe consisted largely of fragments of rhabdomeres, we have extracted a photosensitive pigment with $\lambda_{max.}$ 520 mμ. The chromophore of this pigment is retinene$_1$. We therefore consider it to be a rhodopsin. On exposure to light it bleaches to a mixture of retinene and opsin. This behaviour, characteristic of vertebrate rhodopsins, distinguishes the *Limulus* pigment sharply from certain other invertebrate rhodopsins—squid, octopus, cuttlefish, lobster—which under physiological conditions do not bleach but yield in the light relatively stable red-orange metarhodopsins, in which retinene remains attached as chromophore to opsin[4,6].

The curve shown in Fig. 1 is a difference spectrum measured in the presence of hydroxylamine. This reagent combines with retinene$_1$ to form retinene$_1$

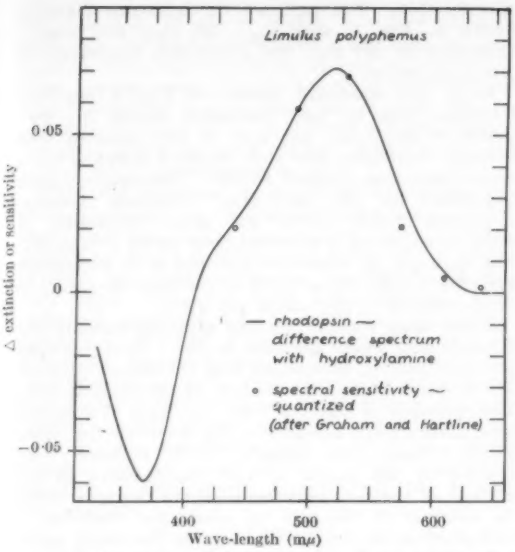

Fig. 1. Absorption spectrum of *Limulus* rhodopsin, compared with the spectral sensitivity of the photoreceptors *in vivo*. The difference spectrum, measured in the presence of hydroxylamine, shows the changes in absorption caused by exposure to light. The positive changes ($\lambda_{max.}$ 520 mμ) represent the bleaching of the visual pigment, and are equivalent to its true absorption spectrum at wave-lengths longer than 450 mμ. The negative changes represent the formation of retinene, oxime ($\lambda_{max.}$ 367 mμ) as a product of bleaching. The absorption spectrum of the visual pigment in solution agrees with the spectral sensitivity of the eye measured electrophysiologically by Graham and Hartline

oxime, with $\lambda_{max.}$ at 367 mμ, which appears as a minimum in this figure. The use of hydroxylamine identifies the product of bleaching unequivocally as retinene$_1$, and also makes the difference spectrum equivalent to the true absorption spectrum at wave-lengths longer than 450 mμ (ref. 7).

The points in Fig. 1 show the measurements by Graham and Hartline of the spectral sensitivity of photoreceptors in the eye of *Limulus*[8]. They are in good agreement with our absorption spectrum.

Our first attempt to find a visual pigment in the *Limulus* eye involved large, sexually mature animals (about 12–15 cm. across the carapace), collected on the beaches while mating. The eyes of these animals were highly sclerotic, and in most cases the corneas were virtually opaque, frequently heavily encrusted with algae, and sometimes perforated. On extracting these eyes, we found no photosensitive pigment or retinene, but obtained only a small amount of chlorophyll, presumably from the algae, and some carotenoid.

We therefore began working with young *Limulus*, 3–5 cm. across the carapace, having pliable, amber-coloured shells, and transparent corneas. The animals were dark-adapted over-night and all procedures carried out in dim red light. A cut was made around the cornea, and the eye was lifted off. The eyes were collected on dry ice and stored at −15° C.

In an earlier preparation of rhodopsin from lobster eyes[4] it had proved advantageous to isolate the structures containing the visual pigment—presumably the rhabdomeres—by using the methods ordinarily employed to isolate the outer segments of vertebrate rods and cones. We hoped in the same way to separate the rhabdomeres of the *Limulus* eye from the remaining tissues and black screening pigment.

In one of our first experiments, the eyes from 44 animals were thawed and ground thoroughly in a mortar with 8 ml. of 45 per cent sucrose in neutral *M*/15 phosphate buffer. This suspension was layered over in a centrifuge tube with neutral phosphate buffer and spun in a Spinco preparative ultra-centrifuge at 40,000 r.p.m. for 10 min. A fine layer of tissue fragments, presumably including the rhabdomeres, collected at the interface between the sucrose and buffer solutions, the remaining tissues and black pigment forming a sediment at the bottom of the tube. The interfacial material was drawn off with a syringe and the sediment was reground and fractionated as before. The interfacial material from both these flotations was pooled, diluted with buffer, and sedimented by centrifuging at 40,000 r.p.m. for 15 min. The faintly straw-coloured supernatant was discarded and the tissue was extracted with 0·6 ml. of 2 per cent aqueous digitonin for 1 hr. at 5° C. After clearing this extract by centrifuging, its absorption spectrum was recorded in a Cary spectrophotometer. On exposure of this solution to light, a just perceptible bleaching occurred in the visible spectrum, accompanied by a small rise of extinction in the near ultra-violet, suggesting the bleaching of a visual pigment to retinene. However, the changes were much too small to yield a reliable record.

We first thought that our isolation procedure had possibly lost most of the visual pigment, and decided therefore to see whether retinene could be obtained from whole eyes and in what quantity. The eyes of 51 animals were ground in a mortar with about three times their volume of anhydrous sodium sulphate. The dry product was pre-extracted with petroleum ether in the dark. The extract contained a yellow carotenoid, epiphasic on partition between petroleum ether and 90 per cent methanol, both before and after saponification. It possessed absorption bands in hexane at about 425, 449, and 476 mμ and was apparently β-carotene. No vitamin A or retinene was found in this extract.

The tissue was then extracted exhaustively in the light with acetone containing a few per cent ethanol and water. The light should have bleached any visual pigment that was present, the acetone-ethanol should have destroyed it by denaturation. The extract, transferred to 2 ml. hexane, was pale yellow, owing to a small residue of carotene. The direct spectrum showed little but general absorption rising steeply into the near ultra-violet. The extract was transferred to 0·3 ml. chloroform, and 0·25 ml. of this solution was mixed with 1 drop of acetic anhydride and 0·5 ml. of saturated antimony trichloride in chloroform. The absorption spectrum, recorded immediately, is shown in Fig. 2, curve 1. It displays the absorption band characteristic of the antimony chloride product of retinene, with $\lambda_{max.}$ 667 mμ, distorted by a second band at shorter wave-lengths ($\lambda_{max.}$ about 600 mμ) caused by some unidentified contaminant. The latter band faded rapidly, so that a second spectrum, recorded a few minutes later, displayed the retinene band alone (curve 2). From curve 2 one can calculate that this extract from 102 eyes contained about 4×10^{-4} μmole retinene, that is, about 4×10^{-6} μmole per eye. This amount of retinene from the whole eye is in reasonable agreement with the amount of rhodopsin we had extracted from the tissue isolated by flotation in the previous experiment. There was no reason to doubt, therefore, that we had isolated the correct tissue ; but

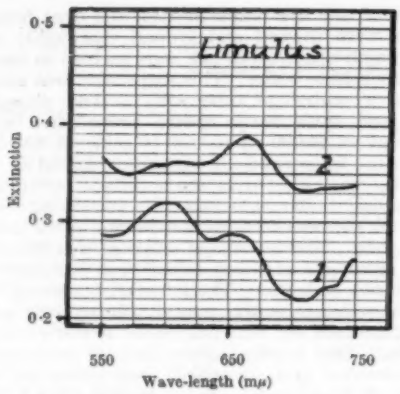

Fig. 2. Absorption spectrum of the product of the antimony chloride reaction with an extract of *Limulus* eyes. The spectrum, measured immediately after mixing the reagents (curve 1), displayed the absorption maximum characteristic of retinene$_1$ in this reaction ($\lambda_{max.}$ 667 mμ), distorted by another maximum at about 600 mμ, due to an unidentified contaminant. The latter faded rapidly, so that the spectrum, re-measured a few minutes later, displayed the retinene band alone (curve 2)

the small amount of photosensitive pigment in the *Limulus* eye introduced special difficulties.

We next isolated the rhabdomere tissue from 378 eyes of young animals, by the flotation procedure described above. This time the isolated tissue was frozen-dried, and pre-extracted exhaustively with petroleum ether. The extract contained β-carotene, about one-third as much as had been obtained from whole eyes.

The residual tissue was extracted with 0·6 ml. of 2 per cent digitonin. The extract was cleared by centrifuging and divided between two micro-absorption cells. Spectra were measured in the Cary model 11 recording spectrophotometer, with a 0–0·1 slide wire that magnifies the extinction 10 times. The results of these measurements are shown in Fig. 3.

The two halves of the extract were first balanced against each other to yield a base line (curve 1). One sample was then irradiated for 2 min. with orange light and the spectrum recorded (curve 2). The record made in these circumstances is a difference spectrum, the difference in absorption between the dark and irradiated solutions. Changes above the base-line represent bleaching due to light, changes below represent the formation of new absorbing material. Curve 2 shows bleaching maximal at about 530 mμ, with the simultaneous formation of a product absorbing maximally at about 370 mμ. This extract was then re-irradiated for 7 min., and allowed to stand in the dark for about 15 min., before recording curve 3. The maximum had now moved to about 520 mμ, an indication that *Limulus* rhodopsin, like that from other species, bleaches over orange-red intermediates such as metarhodopsin, which disappear slowly and thus distort the early difference spectrum. As with vertebrate rhodopsins, however, these distortions are abolished by using hydroxylamine (cf. Fig. 1).

To the remaining portion of the extract, kept in darkness until this point, neutral hydroxylamine was added to a final concentration of 0·04 *M*. Its spectrum was balanced in the spectrophotometer against that of the previously bleached solution, so as to yield a base line. Then this extract was irradiated for 2 min. with white light and left for half an hour in the dark until entirely stable. The result was the difference spectrum shown in Fig. 1, with $\lambda_{max.}$ 520 mμ, and with a minimum at 367 mμ, the absorption maximum of retinene$_1$ oxime.

From the spectrum shown in Fig. 1 one can estimate that we had extracted about 3×10^{-6} μmole of rhodopsin per eye, if one assumes that *Limulus* rhodopsin, like that of other animals, has a molar extinction of about 40,000[7]. This assumption is supported by the fact that bleaching *Limulus* rhodopsin yields about the same extinction of retinene oxime as is obtained from cattle rhodopsin[7]. The quantity of extracted pigment is in reasonable agreement with the amount of retinene we found in whole eyes (4×10^{-6} μmole per eye).

From these results one can estimate roughly the extinction of visual pigment *in situ*. It is generally assumed that in compound eyes the visual pigment is restricted to the rhabdomeres of the retinula cells. The rhabdom of *Limulus* takes the form of a central hub, filled by the dendrite of the eccentric cell, from which radiate fins, usually 10 in number, each formed by the fusion of two rhabdomeres at the surface of contact between adjacent retinula cells[9]. In animals of the size we used, the rhabdom is estimated to be about 100μ long, the fins being about 1·5μ wide (0·75μ contributed by each of the two adjacent rhabdomeres which fuse) and projecting about 10μ from the centre. The entire volume of rhabdomere material, therefore, is about $1{\cdot}5 \times 10^4 \mu^3$ (that is, $10 \times 100 \times 1{\cdot}5 \times 10$). Animals of this size have 800–1,200 ommatidia. The volume of rhabdom per eye is therefore of the order of $1{\cdot}5 \times 10^7 \mu^3$

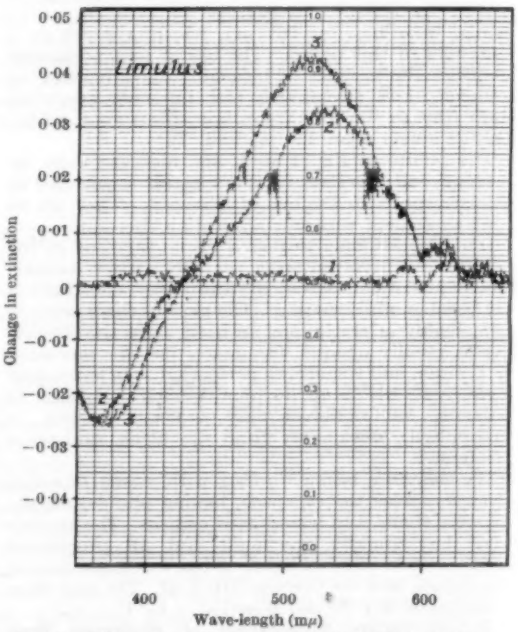

Fig. 3. Difference spectra of *Limulus* rhodopsin in aqueous solution, recorded with a Cary spectrophotometer using a 0–0·1 slide-wire that amplifies the extinction scale ten times. Two portions of the extract were balanced against each other to yield a base-line (1). Then one portion was exposed to light for 2 min.: the difference spectrum (2) shows bleaching maximal at about 530 mμ, and the simultaneous formation of a product with $\lambda_{max.}$ about 370 mμ (retinene$_1$). After a second irradiation and a period in darkness, the solution had become stable (3); $\lambda_{max.}$ now lay at 520 mμ

(ref. 10). If one assumes that *Limulus* rhodopsin has an extinction of about 40,000, 4×10^{-6} μmole of rhodopsin distributed evenly over this volume would have an extinction at 520 mμ of 0·07 in a layer 100μ deep, corresponding to the length of the rhabdom. This extinction is equivalent to an absorption of about 15 per cent. It is interesting that this absorption is of about the same order as occurs in the rods of a number of vertebrate eyes[11].

The only previous report of the extraction of a photo-pigment from the *Limulus* eye is contained in a single sentence and a figure in a paper by Bliss, dealing primarily with squid rhodopsin[12]. According to Bliss, the *Limulus* pigment bleached in the light only after treatment with formaldehyde. The difference spectrum he measured had a maximal extinction of 0·004, lower therefore than one can ordinarily measure accurately; yet the eyes would have had to contain about seven times as much pigment as we find, to yield this extinction under the conditions of his experiment ("15 retinas from 4 cm. wide animals, dissolved in 3 cc."). The $\lambda_{max.}$ of this pigment was at about 480 mμ. This description, therefore, differs on all counts from the present observations.

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FRACTIONATION OF TWO METABOLICALLY DISTINCT CLASSES OF RIBONUCLEIC ACIDS IN ANIMAL CELLS AND ITS BEARINGS ON CANCER

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RECENTLY we have shown[1] that the treatment of suspensions or homogenates of normal and malignant animal cells with aqueous phenol may lead to a fractionation of their ribonucleic acids into two components differing markedly in the nucleotide composition and the rate of uptake of phosphorus-32.

The procedure we are employing for such studies may be summarized as follows. A part of the material labelled *in vivo* or *in vitro* with phosphorus-32-orthophosphate (1–2·5 hr.) was fractionated by the Schmidt–Thannhauser method[2] to obtain the total ribo- and deoxyribo-nucleic acids. The rest of the material was treated with 90 per cent phenol[3], and the ribonucleic acid released into the aqueous phase (fraction *P*) was dialysed against water and hydrolysed with alkali[3]. The phenol layer was treated with ethanol and the precipitated protein material was fractionated by the Schmidt–Thannhauser method to recover the residual ribonucleic acid (fraction *R*). The nucleotide composition of various ribonucleic acids was determined by 'Dowex-1' (formate) chromatography[3]. The separated nucleotides were purified by charcoal adsorption[1], and uridylic acid was further paper-chromatographed in *n*-propanol–ammonia–water (60 : 30 : 10)[4]. Alternatively, the alkaline hydrolysates of ribonucleic acids were first adsorbed on charcoal[4] and then the eluted mixed nucleotides paper-chromatographed as above. Deoxyribonucleic acid was usually extracted with hot dodecyl sulphate[5] from the acid-precipitated residue of the Schmidt–Thannhauser method. The specific activity of ribonucleic acid was calculated as the weighted mean of those of the separated nucleotides, or approximated by determining that of combined adenylic, cytidylic and uridylic acids in the eluate from the unresolved single spot on the paper[4]. The specific activity of deoxyribonucleic acid was determined directly.

The fraction *P* ribonucleic acid had a high guanylic acid content and a low specific radioactivity, while the fraction *R* showed a low guanylic acid content and a markedly high specific radioactivity[1]. The proportion of the fraction *P* in total ribonucleic acid was about 70 per cent in rabbit lymphatic cells and the appendix, about 85 per cent in the rat liver and 90–95 per cent in the mouse Ehrlich ascites carcinoma and rat ascites hepatoma *AH*-7974. The nucleotide composition of various fractions is listed in Table 1. In some instances, especially in tumours, cytidylic acid content was also appreciably lower in fraction *R*.

The most remarkable point of these findings is the fact that the specific activity of the fraction *P* was usually very close to that of deoxyribonucleic acid in various tissues differing markedly in the rate of deoxyribonucleic acid synthesis. Also it must be stressed that, by Kirby's method[6] of ribonucleic acid extraction with phenol which is now extensively employed, one may very well be discarding the bulk of ribonucleic acid with high turnover rate.

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Table 1. Nucleotide Composition of Ribonucleic Acids

Material	Ribonucleic acid fraction	Molar ratio (adenylic acid = 10)			E_{260} recovery (per cent)
		Guanylic acid	Uridylic acid	Cytidylic acid	
Lymphatic cells of rabbit appendix	Total	17·5	10·1	15·3	96·5
	P	20·2	10·6	16·2	101
Rabbit appendix	*P–A*	16·1	10·3	13·0	80·0
	P–B	18·9	10·4	14·6	92·8
	R	15·9	10·0	12·8	95·0
Rat liver	Total	18·0	9·0	16·7	88·2
	P	19·6	10·1	16·0	82·0
	R	14·5	9·0	15·3	79·9
Mouse Ehrlich ascites tumour (7 days)	Total	17·9	10·8	15·1	92·2
	P	19·8	11·1	15·4	98·1
	R	12·1	9·9	11·2	104
Rat ascites hepatoma *AH*-7974 (7 days)	Total	17·4	7·9	15·0	99·2
	P	18·4	11·7	15·1	93·3
	R	11·7	10·0	11·5	100

Table 2. *In vivo* Incorporation of Phosphorus-32 into Fractions of Ribonucleic Acids of some Ascites Tumour Cells

	Mouse Ehrlich ascites carcinoma			Rat ascites hepatoma *AH*-7974
Age of tumour (day)	10	7	7	12
Duration of incorporation experiment (hr.)	2·5	2	2	2
Ribonucleic acid fraction	Specific activity (counts/min./μgm. P)			
Total	15·7	35·0	10·1	9·30
P	6·78	15·0	2·78	4·42
R	65·2	191	48·7	73·6
(Deoxyribonucleic acid)	6·70	17·0	10·1	15·4

The specific activity of the fraction *P* may further be reduced a little by a fractionation utilizing the solubility difference in molar sodium chloride solution. A small amount of soluble ribonucleic acid (fraction *P–A*) with a relatively high specific activity may be removed from the bulk of ribonucleic acid which is precipitated in this salt solution (fraction *P–B*). Such a finding corroborates that of Brown *et al.*[6]. Whether or not the fraction *P–A* is a mixture of the fraction *P–B* and fraction *R* is not clear at the moment.

The simplest interpretation of the above observations would be to postulate the occurrence in animal cells of two principal classes of ribonucleic acids differing in composition, metabolic activity, and mode of linkage with proteins which may serve as the basis of fractionation with phenol. We will call such classes of ribonucleic acids class I and class II, the former being released by phenol and the latter remaining in the residue in typical experiments. It should be emphasized here that the terms fraction *P* and *R* pertain to the extraction procedure using phenol no matter what their chemical or metabolic properties, while the designations 'class I' and 'class II' ribonucleic acids represent partially hypothetical entities.

It is suggested from the specific activity determination that class I ribonucleic acid is duplicated only once during the mitotic cycle like deoxyribonucleic acid, so far as the composition of the cell is kept constant. Because deoxyribonucleic acid is believed to be metabolically rather inert[7], the specific activity of any phosphate compound of cells incorporating radiophosphate, even in the absence of turnover, cannot be appreciably lower than that into deoxyribonucleic acid in the steady state. It can be inferred, therefore, that class I ribonucleic acid may be conserved without turnover in proliferating or non-proliferating cells of fixed composition. The fraction *P* probably contains class I ribonucleic acid in a relatively pure form, being free from contamination by class II ribonucleic acid, which would significantly raise the specific activity of the fraction *P* over that of deoxyribonucleic acid. Whether the fraction *R* represents more or less pure class II ribonucleic acid cannot be determined at the moment.

The close agreement of the specific activities of deoxyribonucleic acid and of fraction *P* ribonucleic acid may not be realized in systems changing their composition. In regenerating rat liver the synthesis of class I ribonucleic acid seems to be enhanced prior to that of deoxyribonucleic acid[1]. In ascites tumour also, the specific activity of fraction *P* ribonucleic acid was sometimes found to be lower than that of deoxyribonucleic acid (Table 2). Indeed, a continuous decrease in ribonucleic acid content per cell during the growth of the Landschütz ascites tumour of the mouse has been reported[8].

The hypothesis of the occurrence of cellular ribonucleic acids in two classes can be tested by studies on ribonucleic acids in subcellular components. However, results so far obtained have been mostly erratic for the reason suggested below, so that we are still not in a position to discuss the matter at full length. To mention some positive results (Table 3), both nuclei and cytoplasm of the rabbit appendix yielded the two fractions which would represent classes I and II. It is noted that the proportion of the fraction *R* was much higher in nuclei the ribonucleic acid of which resembles the class II ribonucleic acid in guanylic acid content and the turnover rate[8]. Also free cytoplasmic ribonucleoprotein particles (ribosomes) of the rabbit appendix gave fractions with properties in line with the results on whole cells.

In earlier experiments consistent results were obtained by an indiscriminate use of commercial phenol, but it was soon found that the use of different batches of phenol might result in a marked change in the efficiency of fractionation. The use of redistilled phenol made the results even worse. Prob-

Table 3. Ribonucleic Acids of some Subcellular Components of Rabbit Appendix labelled with Phosphorus-32 *in vivo*

Ribonucleic acid fraction	Relative amount (per cent of total)			Guanylic acid content (adenylic acid = 10)			Specific activity (counts/min./μgm. P)		
	Nucleus	Cytoplasm	Ribosome	Nucleus	Cytoplasm	Ribosome	Nucleus	Cytoplasm	Ribosome
P–A	1·0	3·0	1·3	—	—	—	—	4·03	—
P–B	43·1	75·6	79·1	17·7	16·4	17·1	7·34	2·24	9·79
R	55·9	21·4	19·6	13·0	12·1	15·0	27·4	5·91	17·5
(Deoxyribonucleic acid)							6·53	2·02	6·31

Nuclei and cytoplasm were separated according to Osawa *et al.* (ref. 2). Ribosomes (free ribonucleoprotein particles) were sedimented at 105,000*g* for 90 min. from a 0·25 *M* sucrose homogenate after removing larger particles by centrifuging at 44,000*g* for 30 min. Values for individual subcellular components were obtained in separate experiments lasting 2 hr.

ably some impurities in the commercial phenol may be of critical importance, and the use of reagents of lower degree of purity generally resulted in a consistent and clear-cut fractionation. The critical factor(s) may also be provided by the tissues. Thus, the rabbit liver gave a relatively 'good' fractionation with re-distilled phenol, while the appendix and kidney yielded a 'poor' fractionation with the same phenol. Some monovalent and divalent cations such as sodium or calcium ions were effective in improving the extent of fractionation, and the addition of ethylenediamine tetraacetate affected the fractionation, while magnesium ions were without effect. Temperature (0–37°) does not seem to be very critical, while the *p*H of the medium (before addition of phenol) is obviously of great importance, the best fractionation so far being obtained at *p*H 6–7. Unless such factors are carefully controlled, isolated subcellular components tend to yield a poor fractionation, and the specific activity of the fraction *P* may no longer be close to that of deoxyribonucleic acid and vary considerably among different subcellular fractions[9].

Several authors[10] have reported a remarkably high guanylic acid content of neoplastic ribonucleic acids. This would suggest a depletion of class II ribonucleic acid in tumour cells, and our observation confirmed it. Extensive uptake of labelled precursors by ribonucleic acid of tumour cells, in spite of the deficiency of class II ribonucleic acid with high turnover rate, may partly be explained by an active net synthesis of class I ribonucleic acid.

It was also reported that the bulk of ribonucleic acid of tumour cells showed little turnover[11]. This fact would again be a reflexion of the marked preponderance in tumour cells of class I ribonucleic acid which appears to be metabolically rather inert. In this connexion it is of interest that the acid ribonuclease activity per unit amount of ribonucleic acid is much lower in tumour cells than in normal cells[12]. However, whether this fact has some bearings on the metabolic stability of the bulk of ribonucleic acid in cancer cells is not clear.

Thus, the relationship between the synthesis of class I ribonucleic acid and that of deoxyribonucleic acid, and the role of two classes of ribonucleic acids in the transfer of genetic information and in protein synthesis may become attractive problems in cell physiology and cancer research.

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CHLOROPHYLL CONTENT OF GIBBERELLIN-TREATED WHEAT SEEDLINGS

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INCREASED plant growth stimulated by gibberellic acid is generally accompanied by a paling of the leaves[1–3]. In a recent review, Brian summarized the situation as follows: "The leaves formed after treatment are noticeably paler in colour than usual. This is a general response to gibberellic acid; it can be to a considerable extent reduced by ensuring that mineral nutrients, especially nitrogen, are not limiting, but even under the best nutrient conditions some chlorosis is usually visible. It is not known whether the chlorosis is due to a destruction of pigment, reduced rate of synthesis of pigment, to a failure of pigment synthesis to keep in step with the increased cell extension, or to change in pigment composition"[4]. In this article we present the results of an investigation of the relation between the effects of gibberellic acid treatment on growth and on pigment content.

'Gibrel' (potassium salt of gibberellic acid) was kindly given by Merck and Co. Wheat grains were germinated and the seedlings grown in Petri dishes containing water or 3×10^{-4} *M* gibberellic acid ($\sim$ 115 p.p.m.), as described previously[5], under 400–500 ft.-candles of white light during 16-hr. days. Chlorophylls *a* and *b* were analysed according to "Official Methods of Analysis" of the Association of Official Agricultural Chemists[6] or by Röbbelen's method[7]; total carotenoids were estimated by the latter method. Twelve to sixteen seedlings were used in each determination.

Results of a typical experiment are given in Table 1. Six days after sowing, the height of plants treated with gibberellic acid had increased at least 60 per cent more than the controls and the leaves were indeed paler. But the chlorophyll content was not

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† Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

Table 1. Influence of Gibberellic Acid on the Growth and Chlorophyll Content of Wheat Seedlings

Treatment	Age 6 days	12 days	18 days*	24 days*	30 days*
			Height (mm.)		
Control	59·5	100·3	140·1	249·0	294·8
Gibberellic acid	98·8	176·1	258·7	356·3	404·5
			Fresh weight (mgm.)		
Control	33·9	75·7	124·3	340·5	544·4
Gibberellic acid	42·2	80·1	131·4	309·3	420·8
			Total chlorophyll (μgm.)		
Control	26·8	56·7	126·0	381·2	583·8
Gibberellic acid	26·1	46·2	81·2	248·6	370·8

* Plants removed from Petri dishes and transplanted to pots of soil after 12 days.

significantly different in treated and untreated plants. After 12 days, the chlorophyll content of the treated plants was about 20 per cent less than in the controls. Total carotenoid values were 12·5 μgm. per plant for the controls and 10·4 μgm. for treated plants. Hence the reductions in amounts of chlorophyll and carotenoid in treated plants were about the same. The difference in chlorophyll content between the treated plants and controls increases with time.

Twenty determinations of the chlorophyll *a* : chlorophyll *b* ratio in each of the two series of plants showed 75·6 per cent of the total chlorophyll consisted of chlorophyll *a* in the controls and 75·5 per cent in the plants grown in gibberellic acid. Obviously, the difference between the appearances of the two groups of plants cannot be related to a change in the proportion of the two chlorophylls.

Leaves of etiolated seedlings grown for 8 days in water in darkness were excised, placed in vials containing water or gibberellic acid, and exposed to 1,000 ft.-candles of light from a water-cooled source similar to that described by Withrow and Elstad[6]. Measurements of chlorophyll content during the subsequent 48 hr. (Fig. 1) showed no significant difference in rate of chlorophyll synthesis during greening, and therefore no difference in chlorophyll anabolism from gibberellic acid treatment.

Five-day-old seedlings grown in water were placed in 3×10^{-4} *M* gibberellic acid and at the same time transferred to continuous darkness. The disappearance of chlorophyll in dark-grown plants and in plants similarly treated in all respects except for treatment with gibberellic acid were compared. The acid had no effect on chlorophyll disappearance, and therefore no effect on chlorophyll catabolism (Fig. 2), even though the plants responded to the gibberellic acid in darkness. The average heights of the treated and control plants after the seven days in darkness were 180·4 and 132·6 mm., respectively.

The findings with young plants (5–8 days old) indicate that gibberellic acid can exert a pronounced

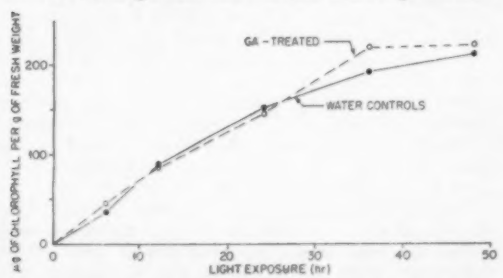

Fig. 1. Effect of gibberellic acid on greening of 8-day-old detached etiolated wheat leaves

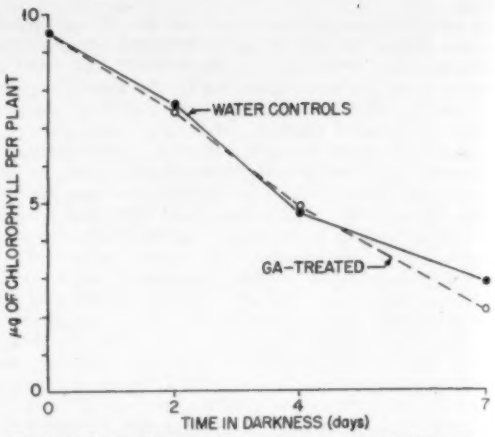

Fig. 2. Effect of gibberellic acid on disappearance of chlorophyll after transferring 5-day-old wheat seedlings to continuous darkness

effect on growth without affecting the total content, anabolism, or catabolism of chlorophyll. The paler appearance of six-day-old treated plants therefore results solely from pigment dilution. Since the pigment content does not differ significantly in these treated and control plants, there is no real chlorosis. The chlorophyll content of older treated plants (12 days or more) is less than in the untreated controls. But since gibberellic acid promotes growth without affecting the content, synthesis, or destruction of chlorophyll in young plants, its influence on chlorophylls and other pigments in older plants is probably an indirect result of its growth stimulation. These results support the suggestion that 'chlorosis' induced by gibberellic acid is in part related to nutritional deficiencies[3,4]. The acid does not influence the pigment content of the very young seedlings adequately supplied with nutriment from the grain, but does reduce the pigment content of older plants. This may be correlated with the growth results in Table 1, which indicate that the untreated plants eventually surpass the treated seedlings in growth-rate.

These results demonstrate that (*a*) chlorosis does not necessarily accompany growth stimulated by gibberellic acid and (*b*) the paling in very young seedlings results solely from a failure of chlorophyll synthesis to keep pace with the increased cell expansion[5]. Besides the simple dilution of chlorophyll, the apparent chlorosis in older plants must also result from delayed effects of malnutrition, not to direct action of the acid on formation or destruction of chlorophyll. Stimulation of growth by gibberellic acid without direct effect on chlorophyll metabolism is consistent with the finding that the acid does not directly affect photosynthesis[9].

We wish to thank Mrs. Helen Luippold, Mrs. Dora Furr, and Mr. Joe White for help in caring for the plants and for assistance in the experiments.

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PROTEIN COMPONENTS FROM TURNIP YELLOW MOSAIC VIRUS

By Dr. J. M. KAPER

Department of Biochemistry, University of Leyden

RECENTLY we reported a degradation method[1] with which soluble protein fragments were obtained from a purified preparation of turnip yellow mosaic virus. Evidence was presented that the alkaline treatment involved had not affected the peptide bonds of the protein. Under the electron-microscope the preparation showed spherical particles of a certain minimal diameter and also larger aggregates of variable size, all of which were much smaller than the virus particles, however.

Since then I have started to investigate the products of the alkaline treatment of the virus by means of electrophoresis and the ultracentrifuge.

Turnip yellow mosaic virus purified by the butanol–chloroform procedure[2] was treated with 0·13 *M* dipotassium hydrogen phosphate–sodium hydroxide buffer of *p*H 11·2 (virus concentration in mixture was 1 per cent). During this treatment, extending over a period of approximately 2½ days at room temperature, the *p*H of the reaction mixture dropped from 11·2 to 11·0. After the reaction, part of the mixture was set aside while the rest was subjected to ultracentrifugation in the Spinco model *L* ultracentrifuge for 2 hr. at 40,000 rev./min. After termination of the run, the resulting pellet and supernatant fractions were separated. The supernatant was set aside, the pellet was re-suspended in distilled water and again ultracentrifuged for 2 hr. at 40,000 rev./min. This washing and ultracentrifugation procedure was repeated once more, after which the pellet was taken up in distilled water. The three solutions (total mixture, pellet, and supernatant fraction) thus obtained were dialysed against 0·1μ *p*H = 7 buffer[3] for 2 days at 5° C. with three additional buffer changes to equilibrate for electrophoretic and sedimentation analysis.

Electrophoresis was performed at room temperature in the Kern *LK*30 micro-electrophoresis apparatus equipped with Jamin interference optics. Sedimentation analysis was carried out at 20° C. in the Spinco ultracentrifuge model *E* with schlieren optics.

Fig. 1 shows the electrophoretic pattern of the reaction mixture after 29 min. of electrophoresis at 53 V. and 3·8 m.amp. The upper limb in the picture represents the mixture of components ascending towards the anode. The presence of three main components with different mobilities is evident, although in the upper limb most of the fastest-moving gradient has left the cell already.

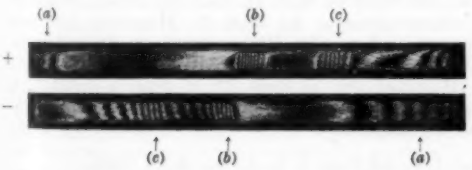

Fig. 1. Electrophoresis pattern of the reaction mixture after treatment of turnip yellow mosaic virus at *p*H 11·2–11·0. Buffer: 0·1μ, *p*H 7, room temperature

(*a*), Polynucleotide gradient; (*b*) gradient of pellet fraction; (*c*), soluble protein gradient

Through analysis of the supernatant fraction (which can be shown to contain the soluble protein[1] and polynucleotide material) and the pellet fraction, gradient (*a*) in Fig. 1 could be identified with polynucleotide material which had been liberated from the virus and which was not dialysable; gradients (*c*) and (*b*) represented the soluble protein and the pellet fraction respectively. Control analysis of intact turnip yellow mosaic virus under the same experimental conditions showed that the pellet fraction and the virus had identical electrophoretic mobilities; this was further corroborated by mixing the two substances and analysing them at *p*H 7·0, 8·3 and 9·5, under which conditions they behaved as one component in electrophoresis. The ultraviolet spectrum of the pellet fraction was predominantly of protein character (maximum 270 mμ, minimum 250 mμ and shoulder at 290 mμ), although it was evident that small amounts of nucleic acid in one form or another were present in the fraction.

Fig. 2*A* shows the sedimentation pattern of the reaction mixture in the ultracentrifuge 5 min. after reaching the desired speed of 59,780 rev./min. Here again we observe three main components and a small amount of a fourth. The identification of the various boundaries with the fractions obtained in the preparative procedure was carried out by separate runs, as in the electrophoresis. In Fig. 2*A* the gradients are designated accordingly. The sedimentation coefficient (uncorr.) of (*b*) was determined from this run and found to be 47 *S*. In separate runs of the pellet fraction at 33,450 rev./min. and at dilutions as much as 0·005 per cent we found sedimentation coefficients of 52–53 *S*. As is evident from Fig. 2*B*, the pellet fraction gives a sharp and symmetrical boundary and is only contaminated by a small amount of substance with sedimentation-rate of approximately 80 *S*. Gradient (*c*) in Fig. 2*A* shows a pronounced polydispersity and represents the soluble protein which we described earlier[1]; (*a*) is the polynucleotide material resulting from the degradation of the virus-nucleic acid. Figs. 2*C* and 2*D* represent the sedimentation patterns of reaction mixtures of turnip yellow mosaic virus treated at *p*H 12·1–11·9 and *p*H 10·8–10·5 (the two *p*H-values indicate the *p*H at the beginning and at the end of the reaction).

The pellet fractions of both mixtures analysed separately had sedimentation coefficients of 52–53 *S* again. The fraction containing the soluble protein from the *p*H 12·1–11·9 treatment (Fig. 2*C*) showed much less polydispersity and the sedimentation

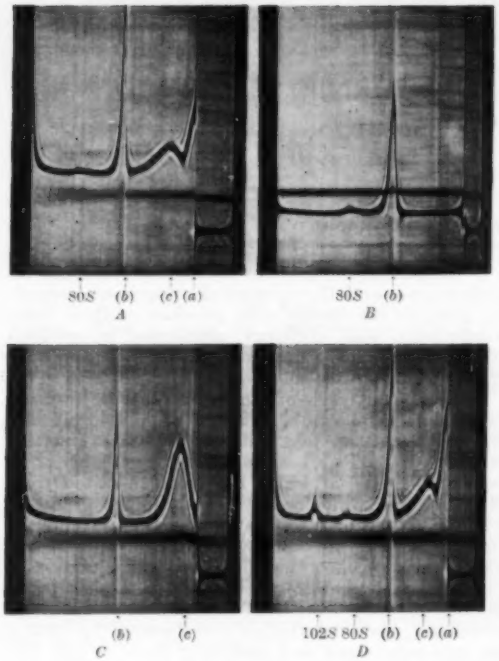

Fig. 2. Sedimentation patterns of degradation products of turnip yellow mosaic virus. Buffers: 0·1μ *p*H 7; temperature, 20° C. (The horizontal streak which is visible on all our schlieren diagrams is not caused by irregularities in the systems under measurement, but could, according to the Spinco field engineer, be attributed to dust particles which had accumulated on the mercury lamp just in front of the slit)

A, reaction mixture from degradation at *p*H 11·2–11·0; 5 min. after reaching speed of 59,780 rev./min.

B, pellet fraction (conc. 0·25 per cent) obtained from degradation at *p*H 11·2–11·0; 26 min. after reaching speed of 33,450 rev./min.

C, reaction mixture from degradation at *p*H 12·1–11·9; 5 min. after reaching speed of 59,780 rev./min.

D, reaction mixture from degradation at *p*H 10·8–10·5; 3 min. after reaching desired speed of 59,780 rev./min.

(*a*) polynucleotide gradient; (*b*) gradient of pellet fraction; (*c*) soluble protein gradient

coefficient determined from the 'peak' of (*c*) was about 11 *S*. Electrophoresis of the reaction mixture of *p*H 12·1–11·9 showed that no change had occurred in the relative mobilities of both components, but that the relative concentrations had changed in favour of the soluble protein. Polynucleotide material could no longer be detected, as apparently the nucleic acid had broken down to dialysable nucleotides. The ultra-violet spectrum of the pellet fraction (Fig. 3) obtained at this *p*H had more protein character; this might be attributed to the absence of the 80 *S*-contamination.

The reaction mixture at *p*H 10·8–10·5 showed quite the opposite picture (Fig. 2*D*) which, of course, could be anticipated. Gradient (*a*), representing the polynucleotides, is again present, gradient (*c*) shows very much polydispersity and, in addition to the 80 *S*-component, there is an appreciable amount of fast-moving substance with a sedimentation coefficient of 102 *S*, which probably represents residual virus. Electrophoresis showed again the gradients (*a*), (*b*) and (*c*) with the same relative mobilities as in the other experiments.

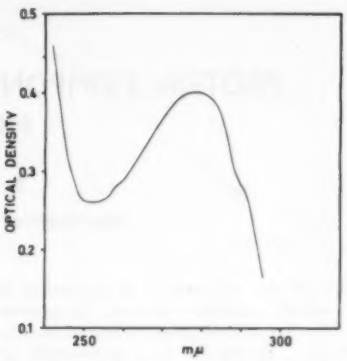

Fig. 3. Ultra-violet spectrum of pellet fraction isolated from treatment of turnip yellow mosaic virus at *p*H 12·1–11·9. Buffer, 0·1*p*, *p*H 7

One main conclusion emerges from the experiments described. Through alkaline treatment of turnip yellow mosaic virus it is possible to penetrate the virus and affect the imbedded nucleic acid in such a way that it starts to leak out in (poly) nucleotide fragments of variable size, depending on the *p*H of the reaction. After the (poly) nucleotides have leaked out, they leave an empty or virtually empty protein shell. This material can be isolated by a simple differential centrifugation procedure. That the protein shell has probably remained intact seems evident when the identical electrophoretic mobilities of both virus and protein are considered and when its sedimentation coefficient, 53 *S*, is compared with those of Markham[4] (48·8 *S*) and of Lyttleton *et al.*[5] (50 *S*) for turnip yellow mosaic virus top component. In addition to this, we have compared the 'natural' virus plus top-component system, isolated by the procedure described by Cosentino *et al.*[6], with a 'reconstructed' system, obtained by mixing the virus (without top component) and the 53 *S* component in approximately the same concentration ratio. The sedimentation patterns thus obtained, with sedimentation coefficients of 52 *S* and 109·5 *S* (virus) for the 'natural' system and 53·5 *S* and 109 *S* for the 'reconstructed' system, were virtually identical.

Besides the full-size protein, fragments of smaller size are obtained[1]. We do not know as yet what is the exact sequence of events during the alkaline treatment; but the experiments described suggest that first the intact protein shells are formed, and they then dissociate into particles of smaller size.

This sequence of events, as well as other aspects of the reaction, are being studied in detail, and I also hope to carry out a complete characterization of both proteins described.

I am indebted to Prof. H. Veldstra for his interest and encouragement, to Miss C. Houwing for her assistance and to the Rheumathology Department and the Medical Chemistry Department of the University Hospital for putting their Spinco model *E* ultracentrifuge and the micro-electrophoresis apparatus at my disposal.

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SULPHASOMIZOLE (5-*p*-AMINOBENZENESULPHONAMIDO-3-METHYL*iso*THIAZOLE): A NEW ANTIBACTERIAL SULPHONAMIDE

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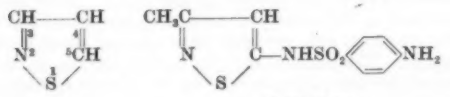
Research Laboratories, May and Baker, Ltd., Dagenham, Essex

THE development of a controlled antibiotic policy, primarily to curb the spread of resistant strains of *Staphylococcus aureus*[1], has led to a renewed interest in sulphonamide therapy. Additional stimulus has also been provided by the introduction of the 'long-acting' sulphonamides, the main advantage of which is probably convenience of administration. The prolonged action of these new products may be attributable to differences in metabolic pathways brought about by differences in substitution in the heterocyclic moieties attached to the N^1 position of the classical structural types. The compounds of this class that have been used clinically during the past few years have all conformed to a traditional chemical pattern, their duration of action and rate of excretion being varied by variation of substituents in known heterocyclic nuclei.

Against this background, it is pertinent to report a new departure in sulphonamide research, in which a hitherto unknown heterocyclic nucleus—*iso*thiazole (I)—has been introduced. The full consequences of this development remain to be worked out, but sufficient work has been completed to show that this new sulphonamide, 5-*p*-aminobenzenesulphonamido-3-methyl*iso*thiazole (the name sulphasomizole has been approved by the British Pharmacopœia Commission), is of considerable interest.

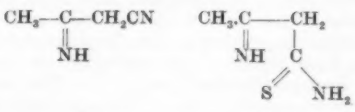


(I) Sulphasomiazole

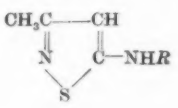
When our work began, we had no experimental evidence that the *iso*thiazole nucleus (I) could exist, but the system had long been of theoretical interest to us because of the similarity between —S— and —CH = CH— in aromatic structures. Thus, the new parent compound should resemble pyridine in many of its chemical properties[2].

We have synthesized mononuclear *iso*thiazoles by two distinct routes. The first involved the oxidation of 5-aminobenz-1,2-*iso*thiazole to *iso*thiazole-4,5-dicarboxylic acid[3], from which 4-*p*-aminobenzenesulphonamido*iso*thiazole was eventually prepared. This compound showed high activity against streptococcal and staphylococcal infections in mice, and the results stimulated us to devise a simpler synthesis of a related 5-sulphonamide.

Treatment of β-imino-*n*-butyronitrile (II) with hydrogen sulphide gave β-iminothio-*n*-butyramide (III)[4], which was oxidized by a variety of reagents to 5-amino-3-methyl*iso*thiazole (IV: $R = H$)[5].



(II) (III)



(IV)

The amine is a valuable intermediate for the preparation of many mononuclear *iso*thiazoles, and more than fifty derivatives of *iso*thiazole have been made[2]. One of the most interesting from the biological point of view is 5-*p*-aminobenzenesulphonamido-3-methyl-*iso*thiazole (IV: $R = SO_2.C_6H_4.NH_2$-*p*)[5], a sulphonamide of high solubility in water (*p*H 6·0, 248 mgm./100 ml.; *p*H 7·0, 2,260 mgm./100 ml.) from which almost neutral, concentrated (more than 50 per cent w/v) aqueous solutions of the corresponding sodium salt can easily be made.

The antibacterial properties of sulphasomizole have been determined by carrying out *in vitro* tests in a synthetic medium similar to that described by Wilson[7]. Aqueous solutions of sodium sulphasomizole were used for these tests. After infection of the medium with a standardized inoculum and incubation at 37° C. for 24 hr., the minimal inhibitory concentrations were recorded.

The organisms included the following: *Staphylococcus aureus* (5 strains), *Streptococcus pyogenes* (2 strains), *Str. agalactiae*, *Str. dysgalactiae*, *Str. uberis*, *Str. pneumoniae*, *Str. equi*, *Str. viridans*, *Str. faecalis* (2 strains), *Alkaligenes faecalis*, *Aerobacter aerogenes* (2 strains), *Escherichia coli* (7 strains), *Klebsiella pneumoniae*, *Proteus vulgaris* (2 strains), *Pseudomonas pyocyanea* (2 strains), *Shigella flexneri*, *Shig. sonnei* (2 strains), *Salmonella typhi* (Rawlings), *S. paratyphi* B, *S. gallinarum*, *S. pullorum*, *S. typhi-murium*, *S. cholerae-suis*, *S. abortus-equi*, *Corynebacterium pyogenes*, *Haemophilus gallinarum*, *Pasteurella septica*

and *Brucella abortus*. Sulphasomizole was active against most of this wide range of Gram-positive and -negative organisms, with the exception of one strain of each of the following organisms: *Str. faecalis*, *Aerobacter aerogenes*, *Shigella sonnei*, *Pasteurella septica*, and two strains of *Escherichia coli*; but other reference sulphonamides, tested under the same conditions, also did not show any activity against these particular strains.

Experiments were carried out in mice infected intraperitoneally with either *Str. pyogenes*, *Str. pneumoniae*, *Staph. aureus*, or *E. coli*. Sulphasomizole was given either by stomach tube at 1, 5, 24, 48 and 72 hr., or graded concentrations were mixed in the diet and fed from one day before to three days after infection. Interpretation of the results was based on two criteria: (1) determination of the median effective dose, from the survival-rates, and (2) the mean effective blood concentration (the concentration of the sulphonamide in the blood necessary for 50 per cent protection of infected mice)[8,9]. Sulphasomizole showed good activity against these experimental infections, and in general appeared as active as the sulphonamides in current use.

The absorption of sulphasomizole into the blood and cerebrospinal fluid, its distribution in the tissues and excretion in the urine were determined in several species. Estimations of the sulphonamide were made by the Bratton–Marshall method[10]. The sulphonamide was given orally or subcutaneously (as the sodium salt). After oral administration to mice, rats and rabbits, it was well absorbed in doses up to 0·5 gm./kgm., and high concentrations of unconjugated drug were produced within half to 1 hr. (It should be emphasized that the term 'unconjugated' refers to the diazotizable material detected by the Bratton–Marshall method before hydrolysis, whereas the term 'conjugated' refers to the extra diazotizable material found after acid hydrolysis. Koechlin, Kern and Engelberg[11] prefer the terms 'directly' and 'indirectly' reacting.) The pattern of the blood concentration curves showed that the drug did not disappear rapidly from the circulation, appreciable amounts being present up to 24 hr. after administration; about 35 per cent was conjugated in the blood.

The sulphonamide was freely distributed in the tissues in rabbits. After an oral dose of 0·5 gm./kgm., maximum concentrations were found in the liver, lung, and spleen within about 2 hr., and at about 4 hr. in the kidney. In pregnant rabbits, sulphasomizole passed freely into the fœtus, and in lactating rabbits, into the milk.

Some information on the blood concentrations in man was obtained from three male volunteers and two patients. A single oral dose of 1 gm. in volunteers produced a maximum blood concentration of about 7·5 mgm./100 ml. within 4 hr., which fell only slightly by 6 hr. and had fallen to 2·0 mgm./100 ml. by 24 hr. About 40–50 per cent of the dose was recovered from the urine in 24 hr. In two patients each given 2 gm. of the sodium salt intramuscularly, the blood concentrations were 11·25 and 20·5 mgm./100 ml. (unconjugated and conjugated drug, respectively) in one patient after 9 hr., and in the other patient 15·7 and 27·2 mgm./100 ml. after 12 hr. These levels were amply maintained by repeated doses of 1 gm. at 12-hourly intervals.

In rats, after a single oral dose of 0·5 gm./kgm., about 25 per cent (of which 70 per cent was in the unconjugated form) was excreted in the urine during the first 24 hr. Excretion from rabbits was a little more rapid, about 35 per cent (of which 50 per cent was unconjugated) being found in the urine during the same period after an oral dose of 0·5 gm./kgm.

Sulphasomizole appeared in the cerebrospinal fluid of rabbits after oral administration of 0·5 gm./kgm. and the concentrations, relative to those found in the blood, were about 10 per cent. Intravenous injection of 0·2 gm./kgm., as a solution of the sodium salt, produced relatively high concentrations in the cerebrospinal fluid, about 85 per cent of the concentration in the blood. The difference between these two results is not fully understood.

Up to the present, we have only examined the concentration of free sulphonamide in the cerebrospinal fluid of one human patient suffering from meningococcal meningitis (James, E., Shiel, M., and Ardley, D. G., personal communication). 9 hr. after 2·0 gm. given intramuscularly as the sodium salt, the concentration in the cerebrospinal fluid was 1·85 mgm./100 ml. (16·5 per cent of the blood concentration). Two days later, after another four intramuscular injections, each of 1 gm., had been given, the concentration in the cerebrospinal fluid was 3·25 mgm./100 ml. 5 hr. after the last dose (17·2 per cent of the blood concentration).

In toxicity experiments, no deleterious effects were observed on the growth-rate of young rats or on the blood picture of guinea pigs, after daily oral doses of 0·5 gm./kgm. for several weeks. There was no significant crystalluria, even though the acetyl derivative of sulphasomizole is only sparingly soluble in water. Metabolic studies are being made to determine the nature of the urinary excretion products, and we have obtained some indication, in rats, that there is a marked increase in excretion of glycuronic acid, and a smaller increase in excretion of ethereal sulphate after continued administration of the sulphonamide.

Intramuscular injections of aqueous solutions of the sodium salt in concentrations as high as 20 per cent w/v are well tolerated in rats; 5 per cent w/v solutions have no deleterious effect when instilled into the conjunctival sac of the rabbit's eye.

To sum up, this new sulphonamide has the attributes of a useful therapeutic agent. It possesses a good all-round antibacterial spectrum and is well absorbed and distributed in the body. It is readily excreted in the urine and animal experiments indicate that it does not produce crystalluria even in high dosage. The sodium salt is highly soluble in water and well tolerated on injection. Further experimental information will be published elsewhere.

Clinical studies of the new sulphonamide are in progress.

We wish to thank Drs. E. James and M. Shiel of the Rush Green Hospital for the opportunity of estimating blood concentrations of this new sulphonamide in two patients.

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LETTERS TO THE EDITORS

PHYSICS

Detection of Recently Produced Fission Products in the Atmosphere

We have been investigating in some detail the trend in fission product-levels of surface air sampled at our laboratory site at Sutton, Surrey, since May 1959. Recently, we detected a sharp increase in the levels of activity, and evidence is presented here which suggests that this was due to the first nuclear weapon test in the Sahara.

The levels of atmospheric radioactivity have been studied by measurements made on dust collected from the air by three systems: (1) a small electrostatic precipitator; (2) a large electrostatic precipitator; (3) a membrane filter assembly. The results of some of our counting measurements are shown in Fig. 1. It will be seen that the increase in activity was pronounced, but transitory, occurring some time during February 28–March 1, 1960.

The activities of the small electrostatic precipitator and the membrane filter assembly samples were measured by β-counting and in Fig. 2 the decay of small electrostatic precipitator sample 83 (February 24–March 1, 1960) is shown. The initial rapid decay is due to daughter nuclei of naturally occurring radon and thoron. After this initial decay, a half-life of 9–12 days is observed for the next 6 days, after which the curve tends to flatten out.

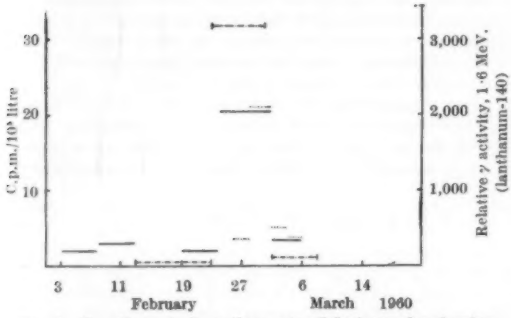

Fig. 1. Counting-rate from three sets of dust samples showing rise between February 28 and March 1, 1960. Horizontal lines indicate period of collection. Membrane filter, ... (left-hand scale); small electrostatic precipitator, ——— (left-hand scale); large electrostatic precipitator, —·— (right-hand scale)

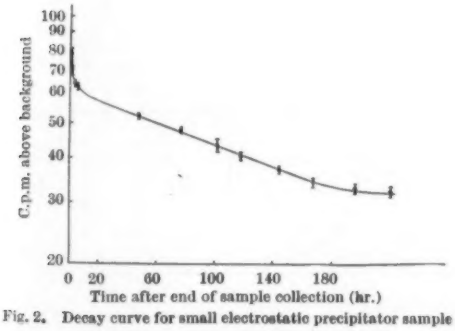

Fig. 2. Decay curve for small electrostatic precipitator sample 83

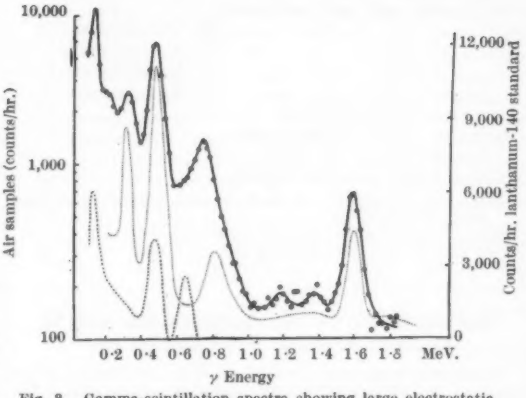

Fig. 3. Gamma-scintillation spectra showing large electrostatic precipitator samples 11(– – – –) and 12 (—○—○) compared with the spectrum of lanthanum-140 (.....)

Fig. 3 shows a γ-spectrum of large electrostatic precipitator sample 12, collected over the period of increased activity, together with a spectrum of large electrostatic precipitator sample 11 taken during the preceding 10 days. The spectrum of a standard source of barium-140 plus lanthanum-140 is also shown. It will be seen that the spectrum of our standard has four prominent peaks[1] and that three of these appear in the spectrum of sample 12. The peak at about 750 keV. is probably due to zirconium-95 plus niobium-95 which would mask the lanthanum-140 line at 815 keV.

If this material is assumed to have come from the nuclear test on February 13, 1960, this would mean that our first measurements were made 20 days after fission. The principal fission products at that time are barium-140, lanthanum-140 and præsodymium-143, which decay with half-lives around 13 days, and cerium-141, with a half-life of 32 days[2]. The decay curve shown in Fig. 2 is very close to that to be expected from such a mixture of activities. Further, the principal γ-lines above 200 keV. will be those due to lanthanum-140, zirconium-95 and ruthenium-103. Energies corresponding to the first two of these nuclides are present in the spectrum of sample 12. The lines of ruthenium-103 would be masked by those of the lanthanum-140 spectrum. These two main lines of evidence combine to demonstrate the occurrence of recently produced fission products in the atmosphere, and it appears likely that these were derived from the French nuclear test.

The concentration of lanthanum-140 in the air was estimated to be 2×10^{-16} c./litre, averaged over the two-day period during which the increase in activity occurred (maximum permissible concentration for a life-time irradiation of occupationally exposed workers, 5×10^{-11} c./litre)[3]. From the known fission yields[2], the concentration of other nuclides can then be estimated. For example, the iodine-131 level would be about 1×10^{-16} c./litre on the same date. There are in all about ten nuclides with activity of the same order of magnitude at this time, so that the total activity would not be expected to exceed 2×10^{-15} c./litre. It is apparent that though detectable, at this

site, none of the nuclides was present at a level having biological significance.

We wish to express our thanks to Prof. W. V. Mayneord, in whose Department the work was carried out, for his help and guidance.

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Radioactivity of the Atmosphere due to Distant Nuclear Test Explosions

The programme at Harwell of rain-water collection and analysis for nuclear debris has been reported by Stewart *et al.*[1-3] and by Crooks *et al.*[4]. In conjunction with this programme, samples of radioactive dust are collected from the atmosphere using aircraft of the Royal Air Force. Similar sampling programmes using aircraft have been reported[5] from the United States, Norway and Sweden.

The dust samples are collected in filters of esparto fibre mounted in holders such that forward movement of the aircraft forces air through the filter. Measurements have been made of the concentration in air of fission-product activities at an altitude of 47,000 ft. in the lower stratosphere and in a composite sample taken between 1,700 and 18,000 ft. in the troposphere.

The filters are analysed for cæsium-137 (30-year) and zirconium-95 (65-day) by a γ-ray spectrometer using a crystal of sodium iodide (3 in. by 3 in. diameter), adequate shielding and a multi-channel pulse analyser. The uncertainty in a single analysis is usually less than 10 per cent provided that, in the case of cæsium-137, the ratio zirconium-95/cæsium-137 is less than 1. If necessary, the samples are allowed to decay before the final determination of cæsium-137.

The stratospheric samples are collected with disk filters (area 45 cm.^{2}) through which the air-flow is measured by an orifice plate assembly and pressure gauges. Typically, about 20 kgm. of air passes through the filter during a flight of 1 hr. between latitudes 50° N. and 56° N. above the United Kingdom. The results of sampling at 47,000 ft. between 1957 and 1959 are expressed in Fig. 1 as the concentration of cæsium-137 in $\mu\mu$c./kgm. of air and also as the ratio of concentrations, zirconium-95/cæsium-137. The collection efficiency of the filters measured at this altitude is nearly 100 per cent. The separate analyses of samples (about 6 per month) are combined to provide a mean monthly concentration.

The concentration of activity in the troposphere is much lower, in accordance with the steep gradient of fission product activity in the atmosphere[1]. In this case samples are collected in cylindrical filters (area 300 cm.^{2}) through which the airflow is measured indirectly. About 1,600 kgm. of air passes through the filter in a flight of 9 hr. along a track over the eastern Atlantic between latitudes 42° N. and 65° N. The flight plan produces a composite tropospheric sample of effective altitude ~4,000 ft. The results are presented in Fig. 2. The activity on the filter is

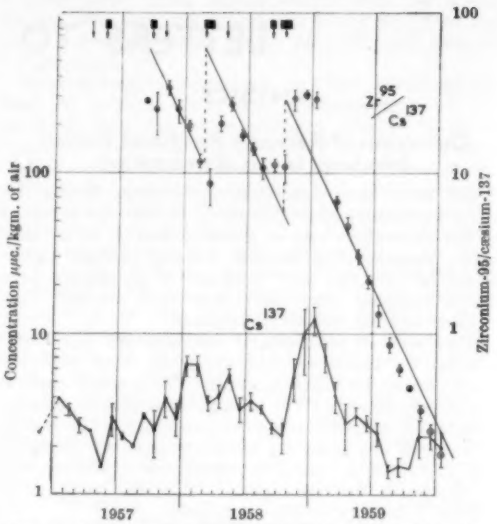

Fig. 1. Concentration of cæsium-137 and zirconium-95 in the lower stratosphere (altitude 47,000 ft.)

corrected by the collection efficiency, measured in these conditions to be 85 per cent. The monthly concentration plotted is the mean of samples from about twenty sorties.

A simple calendar of nuclear test explosions has been fitted to both figures. This has been drawn mainly from results published by Harris[6] and is restricted to explosions of high yield (megaton). It should be recognized that explosions of low yield (kilotons) occurred frequently at other times.

The following observations are based upon deductions from the evidence of Figs. 1 and 2.

The concentration of cæsium-137 in the troposphere follows a seasonal variation, exhibiting peaks in the spring, about May, and troughs in the autumn. This is similar to the effect observed in rain-water by

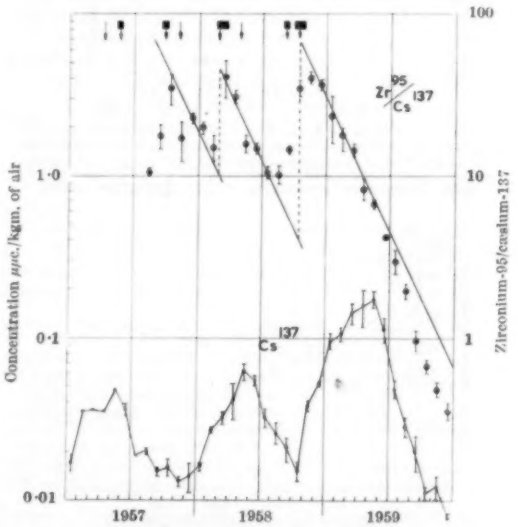

Fig. 2. Concentration of cæsium-137 and zirconium-95 in the troposphere (effective altitude ~ 4,000 ft.)

Stewart *et al.*[2,3]. On the other hand, the record of cæsium-137 concentration in the lower stratosphere (Fig. 1) does not follow the same seasonal variation. During 1957–59 a peak appears in January that may represent a seasonal movement of debris injected in the preceding months.

The ratio zirconium-95/cæsium-137 is a useful index of the age or origin of the mixture of fission products. Within a few months after a new series of test explosions the disturbed conditions are followed by a 'settled period' during which this ratio decays fairly regularly. In some settled periods this decay-rate corresponds to the radioactive half-life of zirconium-95 (65 days), as drawn in the diagrams. Such correspondence implies good mixing, in the regions of sampling, of new fission products from the recent explosions with those from previous explosions, that is, samples are taken from a source of radioactive material that is supplied in constant proportions from the 'reservoirs' of new and old debris.

The proportion of new activity may be calculated from the isotope ratios. For example, during January–June 1959 the proportion of activity in the troposphere due to explosions of autumn 1958 was about 45 per cent. This estimate is to be compared with ~60 per cent derived from the ratios strontium-89/strontium-90 in rain-water at Milford Haven[4] and with 40–50 per cent calculated by Ambrosen[7] for rain-water in Denmark. Departure of the plotted ratios zirconium-95/cæsium-137 after mid-1959 from the earlier line of decay (Fig. 2) corresponds to a reduction in this proportion, that is, to a relative increase in the rate of supply of old debris.

The debris sampled in the lower stratosphere after the autumn 1958 explosions shows evidence (Fig. 1) of good mixing in the second half of 1959. Previously in 1959 the behaviour of the zirconium-95/cæsium-137 ratios is again consistent with a relative increase in the rate of supply of old radioactivity.

Differences between the troposphere and lower stratosphere in the durations of the settled periods and the values of zirconium-95/cæsium-137 are significant. The explosions of autumn 1958 have in the latitudes of the United Kingdom produced a proportionately larger increase in the activity in the troposphere than in the lower stratosphere. This is contrary to the situation in the summer of 1958. In the latitudes of the United Kingdom the two regions of the atmosphere have maintained separate identities, as defined by the ratio zirconium-95/cæsium-137 during 1959.

A fuller account and discussion of the concentration of activity in air and rain-water will be published later.

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Isotopic Composition of Potassium

Recent measurements[1–6] of the natural isotopic composition of potassium have yielded values for the potassium-39/potassium-41 ratio ranging from 13·48 ± 0·07 (Nier) to 13·96 ± 0·05 (Omura and Morito). These results are not inconsistent with the presence of variations of 1 or 2 per cent in the potassium-39/potassium-41 ratio for different samples. Earlier measurements, many of which have been summarized by Reuterswärd[3], cover an even wider range, and include numerous reports[7–12] of differences of 1 per cent or more between the potassium-39/potassium-41 ratios of minerals, normal and cancerous human tissues, and other biological samples. More recently, Voshage and Hintenberger[13] have shown that potassium of abnormal isotopic composition can be produced naturally as a result of the action of cosmic rays on iron meteorites.

The ratio potassium-41/potassium-40 was measured by Nier[1] for a sample of mineral origin and found to be 578 ± 6. Subsequent measurements on other mineral samples have agreed with this figure within 1 or 2 per cent. Fenn *et al.*[14] have reported a difference of approximately 2 per cent in the specific radioactivity of potassium salts from mineral and biological sources. This implies a corresponding difference in the relative isotopic abundance of the radioactive isotope potassium-40. However, other workers[15,16] using a similar technique have found no significant differences between the specific radioactivities of potassium salts separated from various mineral and biological materials.

This communication describes a survey of the isotopic composition of potassium in natural samples. The survey was carried out in two parts. In the first, fifty potassium-39/potassium-41 analyses were made on twenty-seven different samples; in the second, twenty potassium-41/potassium-40 analyses were made on fifteen different samples. Each group of samples included minerals (among them two microclines giving anomalous potassium–argon ages), normal and cancerous human tissues, embryonic tissue, Indian Ocean sea water, two tektites (australites) and one plant sample (*Scaevola spinescens*). Extensive tests were carried out to eliminate sources of experimental error and to establish accurately the probable errors due to the remaining random effects.

Isotopic analyses were carried out with a 60-degree, first-order single-focusing mass spectrometer with a 14-cm. ion path radius. Features of the instrument were the use of a new type of single-filament ion source[17] allowing accurate comparison of isotope ratios, use of dual ion-current collectors and amplifiers[18], and the stabilization of potassium-39 ion currents to allow more accurate measurement of the potassium-40 ion currents[19]. A rotating source assembly allowed rapid interchange of ion sources without changing the vacuum conditions or the adjustment of the ion gun. Chemical separation of the potassium as the tetraphenylboron salt was carried out for each sample, this being converted to the nitrate before analysis.

The repeatability of the results was obtained by repeated analysis of samples from a homogeneous standard material (potassium nitrate, the potassium originating from the Stassfurt sylvine deposit). The probable error for any single analysis was close to 0·3 per cent for potassium-39/potassium-41 ratios,

and 0·7 per cent for potassium-41/potassium-40 ratios.

Investigations were carried out on the effects of gas scatter, non-linearity and temperature sensitivity of the measuring circuits, secondary electron emission from the collectors, ion reflexion from the collectors, stray magnetic fields in the source region, and other factors. In no case did the necessary correction to the potassium-39/potassium-41 ratio exceed 1 part in 1,000.

Tests to determine the effects of impurities in the sample materials showed that, if analyses had been carried out without any chemical purification of the samples, the potassium-39/potassium-41 ratios would have been subject to systematic errors ranging up to 3 per cent. Errors of this magnitude were associated with sample solutions in which only a few per cent of the total number of cations in each sample were potassium ions. A somewhat similar effect has been reported by Reuterswärd[8]. The mechanism of the effect has not yet been definitely established.

Tests to establish the degree to which samples were contaminated by 'normal' potassium were carried out using synthetic control samples of anomalous potassium-39/potassium-41 ratio. Contamination effects were proved to be negligible in all stages of the chemical treatments and in the actual isotopic analysis.

The overall distributions of results for both groups of samples were similar to the random distributions obtained by repeated analysis of the standard KNO_3. None of the samples showed a significant difference in either potassium-39/potassium-41 or potassium-41/potassium-40 ratio relative to the standard. It follows that the assumption of constant potassium-40 relative abundance, widely employed in the application of the potassium–argon dating method, is probably valid within very small limits, at least in the case of terrestrial samples. The normal isotopic composition of the australites shows that there is no significant age difference between the potassium from these and terrestrial sources. The normal potassium-39/potassium-41 and potassium-41/potassium-40 ratios obtained for human tissue suggest that claims of abnormal radioactivity of potassium from organic sources may be due to the presence of impurities in the sample materials.

The systematic errors which can apparently arise from the analysis by solid source techniques of samples containing large quantities of impurities may explain many of the previous reports of significant variations in the potassium-39/potassium-41 ratio of natural potassium.

The values obtained for the mean ratios potassium-39/potassium-41 and potassium-41/potassium-40 in the ion beams reaching the mass spectrometer ion collectors from the standard samples were 13·77 ± 0·03 and 576 ± 3 respectively. The quoted errors are probable errors.

It must be noted that mean ion current ratios, although usually assumed equal to the ratios in the sample material, are not absolute ratios. They include the effects of all systematic errors occurring up to the time of arrival of the ions at the collectors. Such errors can only be definitely established by calibrating the instrument with a sample of known isotopic composition. To eliminate all possible sources of error, the calibration sample must be in the same chemical form and of almost the same isotopic composition as the sample materials. No mass spectrometer appears to have been calibrated for potassium in this way, although a calibration with a synthetic argon isotope mixture has been made by Nier[1].

The availability and cost of separated potassium isotopes of high purity are now such that there must be several laboratories in a position to attempt absolute determinations of the isotopic composition of potassium. Existing instruments could be calibrated with accurately prepared gravimetric mixtures of separated isotopes in approximately normal proportions and used to obtain the absolute isotopic abundance ratios. Laboratory potassium salts, in view of the apparent constancy of the isotopic composition of potassium, could then be generally used for the calibration of mass spectrometers and for the measurement of absolute values of isotopic fractionation effects in the various types of ion sources. Such measurements could be expected to contribute substantially towards a better understanding of surface ionization phenomena.

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Electron Spin Resonance in Hydrogen Peroxide – Water Solutions at 90° K. irradiated with Ultra-Violet Light

Electron spin resonance measurements have been made on ultra-violet irradiated hydrogen peroxide – water solutions. The electron spin resonance spectrometer works at 9·35 kMc./s. and uses a conventional phase-sensitive detector system, with a field modulation frequency of 280 c./s.

The hydrogen peroxide – water solutions were frozen at 90° K. in quartz tubes. Solutions of concentrations 46–87 per cent w/w hydrogen peroxide

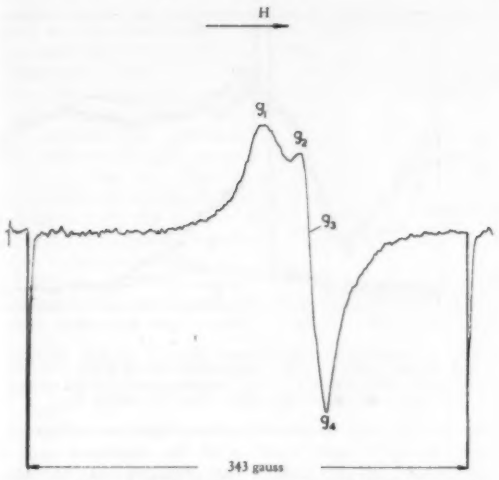

Fig. 1. Primary radical produced by ultra-violet on 87 per cent hydrogen peroxide at 90° K.

formed transparent glasses without much cracking. They were then irradiated for several hours using a Hanovia mercury vapour lamp. The ultra-violet light was filtered by the 'Pyrex' glass Dewar holding the frozen samples so that only wave-lengths longer than approximately 3000 Å. were transmitted.

The samples were quickly transferred to the spectrometer cavity (also at 90° K.) so that no warm-up took place. They then gave the spectrum shown in Fig. 1. The shape of the spectrum is very nearly the same for all the solution concentrations tested —46, 61, 78 and 87 per cent w/w. The g-values for the various points of the spectrum of this 'primary' free radical are given in Table 1. The samples were then warmed up by transferring them to a copper block maintained at a given temperature, storing them there for 5 min., and then returning them to the cavity, which was kept at 90° K. This process was repeated many times for the same samples, raising the temperature of the copper block between each step. On warming to temperatures above 130° K., the primary free radical is converted to another 'secondary' free radical. The spectrum for this radical is shown in Fig. 2. The conversion of the primary radical to this secondary radical is shown in Fig. 3, where $\frac{n_2}{n_1+n_2}$ and n_1+n_2 are plotted against warm-up temperature (n_1 is number of radicals of primary species and n_2 is number of radicals of secondary species). The ratios $\frac{n_2}{n_1+n_2}$ were calculated from the relative heights of the peaks g_1 and g_2 in the various spectra. It is seen from Fig. 3 that radical destruction takes place over the same range of temperature as that in which primary radicals are converted to secondary radicals. Measurements of the temperature for $\frac{n_2}{n_1+n_2} = 0{\cdot}5$ show that this is $130 \pm 5°$ K. for the four solution concentrations tested. The g-values of the various points of the secondary radical are also given in Table 1. The spectrum of the secondary radical shows the g-value anisotropic shape predicted by Searl *et al.*[1] for a polycrystalline sample containing axially symmetric crystals, and which identifies g_1 as $g_\parallel$ and g_4 as $g_\perp$. The spectrum also shows hyperfine splitting of amount 11 ± 2 gauss on the g_1 and g_4 peaks.

Table 1

Species	g_1	g_2	g_3	g_4
Primary	2·0375 ± 0·0008	2·0165 ± 0·0004	2·0105 ± 0·0004	2·0097 ± 0·0004
Secondary	2·0295 ± 0·0010	2·0128 ± 0·0004	2·0096 ± 0·0004	2·0034 ± 0·0004

The occurrence of two separate radicals under these conditions does not appear to have been reported before. Electron spin resonance spectra have been recorded by Kaitmazov *et al.*[2] from ultra-violet irradiation of hydrogen peroxide – water solutions at 77° K. without warming the sample above this temperature. They found a spectrum which showed

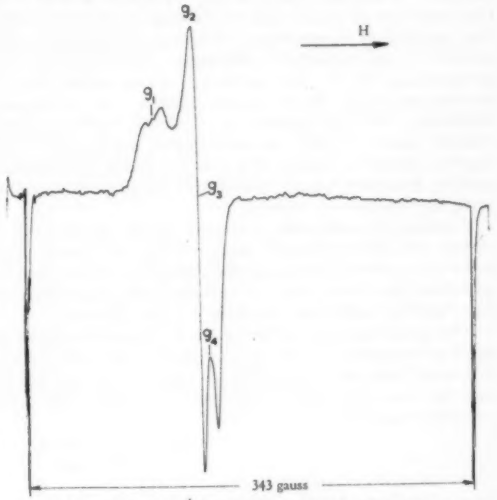

Fig. 2. Secondary radical produced by ultra-violet in 87 per cent hydrogen peroxide at 90° K., warmed up to 138° K., and then measured at 90° K.

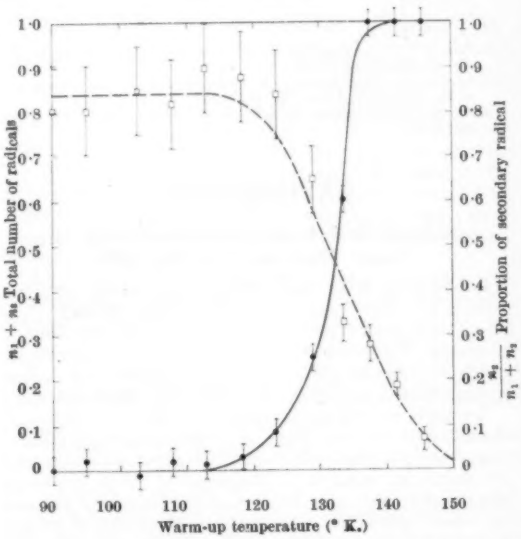

Fig. 3. Plot of $\frac{n_2}{n_1+n_2}$ (●) and n_1+n_2 (□) against temperature of warm-up for 87 per cent hydrogen peroxide

the same g-values as the primary radical above, but which also showed a hyperfine splitting of 12 gauss on the g_4 peak. It therefore appears that their samples contained a mixture of the primary and secondary radicals with the primary radical predominating. The discrepancy between their spectrum and that of Fig. 1 might be explained if Kaitmazov *et al.* used ultra-violet wave-lengths shorter than 3000 Å. Hodgson *et al.*[3] also performed electron spin resonance measurements on a 40 per cent solution irradiated at 77° K. with 2537 Å. ultra-violet; but the resolution obtained was not good enough for comparison with the above measurements. It is also quite likely that the spectrum for the primary radical (Fig. 1) contains a small amount of secondary radical spectrum, and this might account for the g_3 shoulder. The secondary radical spectrum has been reported before by Gorbanev *et al.*[4] and also by Ghormley *et al.*[5], using the method of dissociation of hydrogen peroxide and water in an electrical discharge and freezing the products at 77° K. The spectrum shown in Fig. 2 is in agreement with these measurements; in particular Ghormley *et al.* quote $g_1 = 2{\cdot}027$, $g_3 = 2{\cdot}0085$, which agree within experimental error with the values given in Table 1. We have also performed similar discharge experiments and obtained agreement with the secondary radical.

Further experiments are now being performed in order to obtain more information on these two radicals, and the results of these measurements will be published in due course. It is hoped that this information will be sufficient to identify the radicals.

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RADIOPHYSICS

Ionospheric Effects associated with the Solar Flare of July 10, 1959

On July 10, 1959, the Sydney Observatory reported a class 3+ solar flare lasting from 0210 to 0410 U.T. The Cable and Wireless, Ltd., Receiving Station at Singapore also experienced a sudden fade-out of all radio circuits at 0210 U.T., the circuits not being re-established until between 0415 and 0430 U.T.

Ionospheric changes associated with the flare were observed at the Department of Scientific and Industrial Research Radio Research Substation at Singapore by using an automatic ionosonde[1]. Fig. 1 shows measured values of f_0F2 and of the lowest frequency on which reflexions were obtained from the E or the F layer (f min.), together with median values for the same parameters for control days (July 1–20). A large

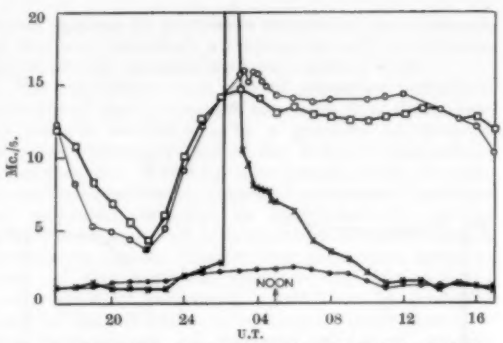

Fig. 1. Ionospheric characteristics observed on July 10, 1959, and control days. ○—○, Measured values f_0F2; ×—×, measured values f min.; □—□, median values f_0F2 for control days; ●—●, median values f min. for control days

change in f min. occurred between 0200 and 0230 U.T., and this is in agreement with the reported time of occurrence of the flare. The changes may be compared with those observed during the solar flare of February 23, 1956[2,3].

An approximation to the increase in the electron density in the D layer has been computed from measurements of f min. and is presented in Fig. 2 as a ratio:

$$B/B_0 = (f1 + fL)^2/(f2 + fL)^2$$

where fL is the gyrofrequency around the vertical component of the geomagnetic field, $f1$ is f min. on July 10, 1959, $f2$ is f min. on control days (median).

A pulse in the measured values of f_0F2 was also observed immediately after the period of very high absorption. This pulse is similar to that studied by Minnis and Bazzard[2] and the same technique has been used to form an estimate of the amplitude of the pulse. On this occasion the best measured value of f_0F2

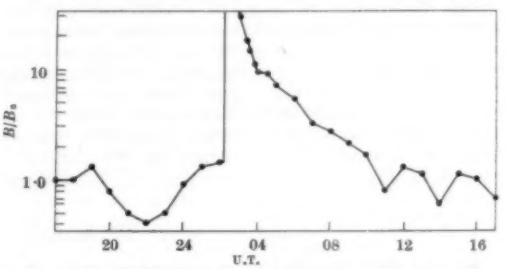

Fig. 2. Ratio of D-layer ionization on July 10, 1959, to median value of that observed on control days

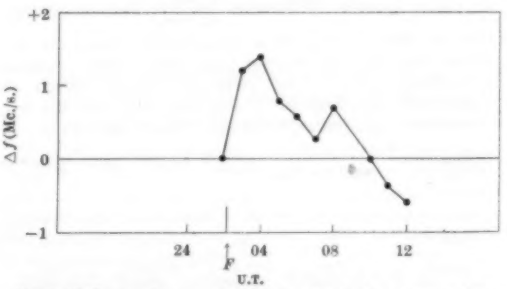

Fig. 3. Difference between measured and probable values of f_0F2 on July 10, 1959, and control days. F, Time of commencement of flare

prior to the disturbance was at 0200 U.T., and the most probable values for subsequent hours have been derived from this value and results recorded on the control days. The differences, Δf, between the measured and probable values of f_0F2 at each hour are shown in Fig. 3. The pulse reached its maximum amplitude between 0300 and 0400 U.T., the time of day in July when f_0F2 normally reaches its maximum value at Singapore.

Differences between the measured and probable values for all hours between 0200 and 1200 U.T. on each day in July have therefore been examined for similar pulses but none was found. The enhanced ionization of the $F2$ layer on July 10 can therefore reasonably be attributed to the effects of the solar flare.

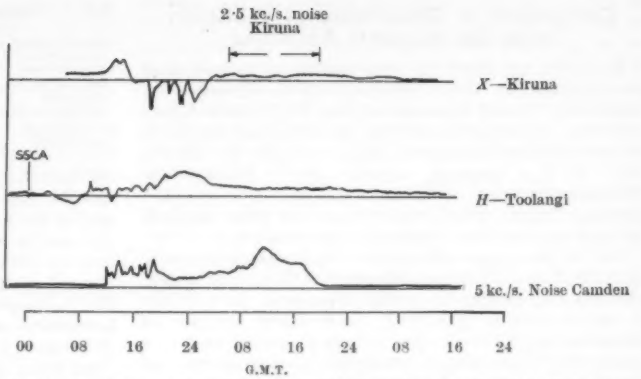

Fig. 1. Comparison of geomagnetic and very-low-frequency noise records (December 4, 1958) from Kiruna, Sweden, and Camden, New South Wales

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Observations of Atmospheric Radio Noise

RECENT observations of radio noise from the upper atmosphere mainly at a frequency of 5 kc./s. have shown that it appears frequently to come from sources of limited geographical size. For example, records of the noise amplitude at places only 1,000 km. apart differ considerably in their time variations, while directional measurements of the apparent noise sources have suggested that they are sometimes as small as 500 km. in extent[1-3].

Yet, although the illumination of the Earth by this radiation in the Australian area appears to be both patchy and sporadic, its occurrence is clearly associated with world-wide disturbances of the geomagnetic field. It is therefore of some interest to examine any evidence for a world-wide noise pattern. Aarons, Gustafsson and Egeland[4] have reported observations of radio noise in the kilocycle region at Kiruna, Sweden. They detected the radiation mainly in two bands centred at about 750 c./s. and 2·5 kc./s. between the hours 0600 and 1900 G.M.T. on December 5, 1958.

Examination of routine records of radiation amplitude in the band 4 kc./s.–6 kc./s. obtained at Camden, New South Wales, has shown that following a geomagnetic sudden commencement (S.S.C.) at 0035 G.M.T., December 4, 1958, a noise storm began suddenly at 1250 G.M.T. on the same day and lasted until 2200 G.M.T. on December 5, 1958. As found on many other occasions at Camden its beginning coincided with a bay-like variation in the magnetic record. Fig. 1 shows a comparison of the Kiruna and Camden geomagnetic and noise records. The early part of the noise storm was apparently not recorded at Kiruna although this could have been due to the different recording technique used at the two places.

It therefore appears likely that there is a world-wide pattern of noise illumination during geomagnetic disturbances, although more examples will be needed to confirm this. The study of this pattern may be of considerable importance because of the possibility that it represents an image of the disturbed regions of the outer atmosphere which are the sources of auroral particles. Investigations of whistling atmospherics have led to the belief that they are guided by ionization ducts in the outer atmosphere, probably lying along the geomagnetic field lines[5]. These magneto-ionic ducts will also guide any radiations in the kilocycle frequency band which are generated in the outer atmosphere and will thus project an image of the radiation sources on to the ionosphere. The radiation itself is likely to be caused by emission from fast charged particles traversing the plasma of the outer atmosphere.

Future studies of the world image at 5 kc./s. may be made either with a network of ground receivers or with satellite receivers. For an instantaneous picture, ground receivers would be necessary since a satellite takes 24 hr. to scan the whole Earth, whereas the average duration of noise bursts is only about 3 hr. Also, the geographical spacing between successive orbits is considerably greater than the scale of the fine structure of the image so far established[3]. Satellite and ground-based techniques are thus complementary and it is planned at this laboratory to make use of both.

The geomagnetic records of Toolangi Geomagnetic Observatory were supplied by the Commonwealth Bureau of Mineral Resources.

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Correlation of Occurrence of Whistlers with Geomagnetic Activities

In order to study the correlation of geomagnetic activities with the frequency of occurrences of whistlers, I have investigated the I.G.Y. and I.G.C. data for 2 years, that is, July 1, 1957–June 30, 1959, at the stations Toyokawa (geogr. co-ord. 34° 50′ N., 137° 22′ E.; geomag. co-ord. 24·5°, 203·5°) and Wakkanai (geogr. co-ord. 45° 22′ N., 141° 41′ E.; geomag. co-ord. 35·3°, 206·0°) and the 3-hr. range K indices of Kakioka Magnetic Observatory.

To show most effectively the geomagnetically disturbed days, I have defined as 'effective K index' the sum of products of the K-indices 5, 6·5 (for 6 or 7) and 8 (for > 8) and their number of occurrences per day; the indices 5, 6 or 7 and > 8 mean moderate storm, moderate severe storm and severe storm, respectively. At first I drew a diagram showing the correlation between my 'effective K index' and the number of occurrences of whistlers, and found that they can be classified fairly distinctly into two groups: one belongs to the winter group, October (or November)–March (or April), where many whistlers are found every month; the other to the summer group, May–September, where smaller numbers are found. It seems that it is difficult to find a single law applicable both to winter and to summer groups.

It may be deduced that the frequency of occurrences of whistlers has a definite seasonal variation depending on thunderstorm activities, distributions of electron density and geomagnetic intensity in the exosphere and in the ionosphere due to solar activities and other yet unknown cosmic phenomena; daily variations due to effective K index are superposed upon this general seasonal variation. Therefore I investigated the correlation of the effective K index with the frequency of occurrences of whistlers observed at Wakkanai in most frequent periods, that is, during September and December 1957, January, February, March and December 1958, January, February and March 1959; Wakkanai was selected owing to remarkable occurrences of whistlers there.

Table 1. Correlation between Lag of Occurrences of Whistlers and Geomagnetic Disturbances

Lag of days of occurrences of whistlers behind geomagnetic disturbances	−3	−2	−1	0	1	2	3
Coefficient of correlation	−0·11	−0·21	−0·12	−0·25	−0·19	+0·60	+0·20

Taking as reference disturbed days where effective K indices are more than 6, I investigated the correlation with the frequency of occurrences of whistlers, and found that it was most remarkable on the second day after geomagnetic disturbances; really the correlation coefficient on the second day was 0·60, as shown in Table 1, and in Fig. 1, from which I conclude that whistlers occur most frequently on the day which lags two days after geomagnetic disturbances. In the summer group the same correlation as that in the winter was not found.

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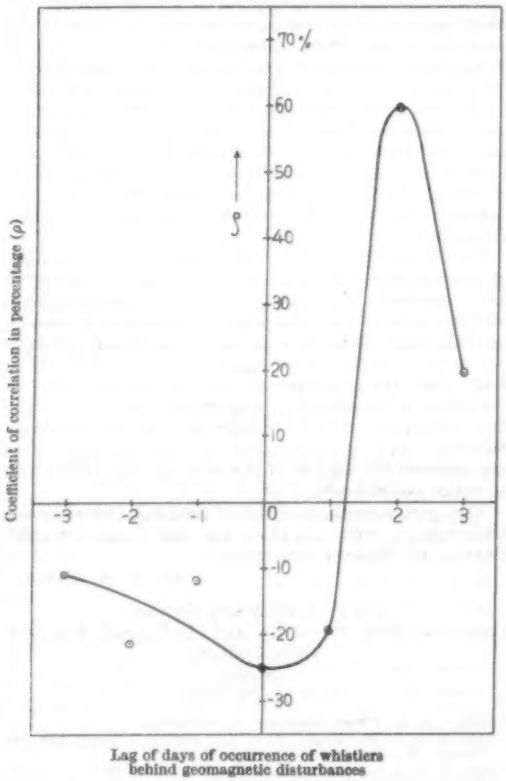

Fig. 1. Correlation between lag of occurrence of whistlers and geomagnetic disturbance

GEOCHEMISTRY

Origin of Chondrules and Chondritic Meteorites

More than 90 per cent of all stony meteorites are classified as chondrites, because of the presence of chondrules—spheroidal aggregates of one or more silicates, commonly olivine and orthopyroxene. In form, texture and structure chondrules are not like any spheroidal bodies observed in terrestrial rocks, and their origin has been argued by investigators of meteorites for more than a century, without a generally accepted solution being reached. Roy[1] has recently provided an admirable summary of the hypotheses of origin. Most of these hypotheses imply that chondrules were derived originally from the crystallization of a silicate melt. These hypotheses are often linked with the generally accepted belief that these meteorites are fragments of a disrupted body of asteroidal or planetary dimensions.

I would suggest an alternative hypothesis, that the structure of chondritic meteorites is the result of reaction and recrystallization of pre-existing material essentially in the solid state, and that many, if not all, chondritic meteorites, instead of being fragments of a disrupted planet or asteroid, have always been independent and individual objects.

The pre-existing material I believe is represented by the carbonaceous chondrites, a small group (19 out of a total of about 800 chondrites), quite distinct

from all other meteorites in chemical composition (they contain carbon, organic compounds, water-soluble compounds, and much combined water) and in mineralogical composition (the silicate material is largely chlorite, a hydrated magnesium–iron silicate). Chlorite decomposes on heating above about 600° C. to give olivine and orthopyroxene; if carbon is present, a natural smelting will take place, reducing some of the combined iron to metallic iron, and thereby increasing the magnesium content of the silicates. The reactions can be represented as follows:

$$\underset{\text{chlorite}}{Mg_4Fe_2Si_4O_{10}(OH)_8} \rightarrow \underset{\text{olivine}}{2(Mg,Fe)_2SiO_4} + \underset{\text{orthopyroxene}}{2(Mg,Fe)SiO_3} + 4H_2O$$

$$\downarrow +2C$$

$$4MgSiO_3 + 2Fe + 2CO_2$$

These reactions will produce the mineralogical composition of the usual chondritic meteorites, an aggregate of olivine and/or orthopyroxene, with some metallic iron. This relationship between carbonaceous chondrites and the other chondritic meteorites was pointed out by Wiik[2], who stated: "This is intended only to give a picture of the chemical relations between the subgroups, and not intended to suggest the genesis". I believe this is not only a chemical relationship but also a genetic one.

The texture and structure of the chondritic meteorites is quite remarkable if they have originated in any body with a considerable gravitational field. Many of them are very porous and friable, so much so that they can be crumbled in the hand, indicating they were not consolidated under pressure. They generally contain nickel–iron in amounts ranging from 5 to 25 per cent, this metal being intimately mixed with the low-density silicate minerals and showing no sign of gravitational segregation. Both these features are readily explicable if these meteorites are the product of chemical reconstitution of carbonaceous chondrites triggered off by heating to 600° C. or higher. A possible explanation is that carbonaceous chondrites represent aggregates of primitive cosmic dust, and that most of them have, during the course of geological time, approached sufficiently close to the Sun to be heated enough to set off the reactions converting them to olivine–pyroxene chondrites.

Suggestive evidence in favour of such an explanation is provided by the age data on chondrites. Gerling and Rik[3] have measured the ages of several chondrites by the potassium–argon method, and found them to differ one from another, ranging from 720×10^6 years up to $3{,}000 \times 10^6$ years. Different ages for different chondrites are to be expected on the hypothesis outlined here, since the potassium–argon age will give the time since recrystallization.

The hypothesis outlined here can be tested if we can devise methods for determining the temperature of crystallization of the minerals making up the chondrites. A few clues are already available. The St. Marks stone contains quartz, and hence its temperature of crystallization (at zero pressure) cannot have exceeded 867° C. The Indarch stone contains tridymite, which, if it crystallized within the field of stability of this mineral, implies a temperature of crystallization between 867° and 1,470° C. Other geochemical thermometers which should be investigated are: (*a*) composition relationships between olivine and co-existing pyroxene; (*b*) the nature of the plagioclase feldspar (present in small amount in most stone meteorites), whether low-temperature or high-temperature; (*c*) the nature of the nickel–iron phases—both kamacite and tenite are present in many stony meteorites, but usually as individual grains, not in Widmanstätten intergrowths. Investigations along these lines are proceeding in this Museum.

If the origin of the chondritic meteorites is that outlined above, the other meteorite types—achondrites, pallasites, and siderites—can be explained as the products of gravitational differentiation of a planetary or asteroidal body formed by the aggregation of chondrites. Such a body would probably pass through a molten stage as a result of the heat produced by aggregation, chemical reactions, and nuclear disintegrations. Given a moderate gravitational field, this would produce a silicate mantle overlying a metallic core. Subsequent disintegration would give rise to the essentially metal-free achondrites, and the silicate-free siderites, the few pallasites coming from a transition zone. In this connexion it should be noted that diamonds have been found only in siderites and achondrites, indicating that only these meteorites crystallized under sufficiently high pressures.

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CHEMISTRY

Pentafluoropyridine

IN two recent communications[1,2], a general method for the preparation of perfluoro-aromatic compounds was described. It involved the defluorination of saturated[2] and unsaturated[1] cyclic fluorocarbons over a metal surface at 400–600°, when the corresponding aromatic fluorocarbons were produced. For example, hexafluorobenzene was obtained[1] from octafluoro-*cyclo*hexa-1 : 3- or -1 : 4-diene, and octafluoronaphthalene[2] from perfluorodecalin; several other examples were also described[2].

We now report the first synthesis of an unsaturated perfluoro-heterocyclic compound by a similar process. The starting material was undecafluoropiperidine, which was prepared by known routes—the electrochemical fluorination of either piperidine[3] or pyridine[3,4]. When passed through a heated nickel tube (2 ft. 6 in. long × 1 in. diameter) packed with nickel gauze, this gave pentafluoropyridine in moderate but significant yields. In a typical experiment, undecafluoropiperidine (3·4 gm.) was passed through the tube at 560° with a nitrogen flow-rate of 4·0 litres/hr. Gas-liquid chromatographic analysis (dinonyl phthalate/kieselguhr : nitrogen carrier gas) of the pyrolysate (2·1 gm.) showed that at least four components were present in addition to some unchanged starting material. The component with the highest retention time was separated from several combined pyrolysates (5·0 gm.) by preparative-scale gas-liquid chromatography[5] and was distilled *in vacuo* from phosphorus pentoxide to give pentafluoropyridine

(0·55 gm.), boiling point 84°, n_D^{20} 1·3834 (found: C, 35·6; C_5F_5N requires C, 35·5 per cent): mass-spectrometry, principal mass peaks: 169, C_5F_5N; 150, C_5F_4N; 138, C_4F_4N; 124, C_4F_4. The compound had $\lambda_{max.}$ at 254 mμ (ε, 2,700 in ethanol). The presence of strong bands in the infra-red spectrum at 1,650, 1,537, and 1,505 cm.$^{-1}$ was also consistent[6] with a pyridine structure, and the absence of strong bands at frequencies higher than 1,650 cm.$^{-1}$ showed that no olefinic bonds were present.

The other three components from the pyrolysis have not yet been identified, but are believed to be unsaturated.

Pentafluoropyridine is a colourless and almost odourless liquid. It is immiscible with water but miscible with organic solvents. We have been unable to detect any basic properties of the compound. A potentiometric titration in aqueous ethanol against $N/20$ acid showed no end-point. Hydrogen chloride in dry ether gave no hydrochloride, and no picrate was formed in ethanol solution. This behaviour is different from that of the primary amine pentafluoroaniline[7], which was weakly basic and formed an easily hydrolysable hydrochloride in ethereal solution. It is noteworthy that pyridine is a stronger base than aniline.

We thank Dr. J. R. Majer for the mass-spectrometric results.

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A Modification for washing the Benzidine Sulphate Precipitate in the Determination of Sulphate

Among the numerous procedures that have been developed for determining sulphate with benzidine, that reported by Kent and Whitehouse[1] is most commonly employed as a micromethod. The reliability of the method has also been confirmed by me.

In this, as in all the other procedures, difficulty is experienced in removing the excess of benzidine from the benzidine sulphate precipitate without losing any of the latter. The difficulty was previously overcome by collecting the benzidine sulphate precipitate in a porous glass filter crucible and washing the unreacted soluble benzidine thoroughly. The method yields accurate and reproducible results provided that the filter is of standard porosity and the filtering time is held constant. Owing to the difficulties associated with the use of the filter crucible, the method is, however, time-consuming.

I have developed the following procedure for washing the benzidine sulphate after its precipitation by the method of Kent and Whitehouse. The contents of the precipitation tube are transferred to a centrifuge tube of about 10-ml. capacity which tapers to a sharp point. One drop of an aqueous suspension of barium sulphate which has been carefully rinsed free of excess barium or sulphate ions is then added and it is centrifuged for 5 min. at 3,000 r.p.m. Two more drops of the barium sulphate suspension are added, and the tube centrifuged for a further 15–20 min. The upper clear layer is then removed and the mouth of the tube dried with cellulose tissue. About 9 ml. of an alcohol–acetone (1 : 1) mixture is added to the tube, and the precipitate is broken up with a stiff steel wire. The tube is centrifuged about 5 min., two drops of the barium sulphate suspension are added, and the centrifugation continued for about 10 min. It should be noted that the total amount of barium sulphate added forms a cone 1–1·5 mm. high at the bottom of the tube when tightly packed. The upper clear layer is decanted, about 9 ml. of the alcohol–acetone mixture is added and the tube is centrifuged for about 10 min. After the supernatant has again been removed, 3 ml. of N hydrochloric acid is added to dissolve benzidine sulphate precipitate, which is broken up with a steel wire. After ½ hr., 2 ml. of water is added to the tube, and the latter is centrifuged for 5 min. The solution containing the benzidine is transferred to another tube for diazotization by the method of Kent and Whitehouse.

The microprecipitate of benzidine sulphate is always enclosed between two tightly packed layers of barium sulphate during the decantations. After the first centrifuged precipitate has been broken up, the benzidine adsorbed on the barium sulphate is removed by washing with the alcohol–acetone mixture. After the centrifuged precipitate has been broken up a second time, the pure benzidine sulphate is dissolved in the hydrochloric acid. The solubility of barium sulphate is so slight that it cannot cause any significant error.

In spite of its apparent complexity, the method can be readily employed in routine analyses. One hundred analyses can be performed daily with a centrifuge that takes 50 tubes and a larger throughput can be achieved by using a larger centrifuge. The accuracy of the method is about the same as reported by Kent and Whitehouse. The absorbance reading for the blank is 0·020 when a Beckman *DU* spectrophotometer is employed.

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Freezing-Point Depressions of Hoagland's 'Carbowax' Systems

Solutions of various osmotic pressures have been widely used in biology. Sugars and their derivatives, commonly used to maintain osmotic pressures, have the drawback that many, if not all, of these compounds can be metabolized by the test tissue or organism. McClenden and Blinks[1] described the use of 'Carbowax 4000' (polyethylene glycol) in maintaining various osmotic concentrations in a study of the Hill reaction. Excellent results were reported. This

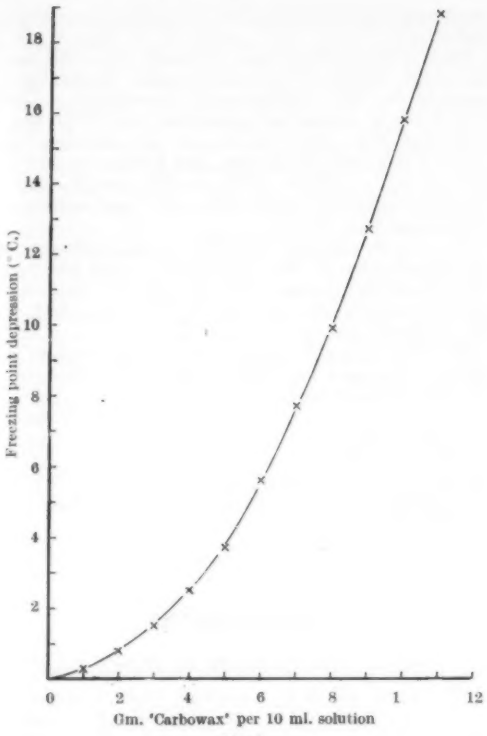

Fig. 1

laboratory has successfully used 'Carbowax 4000' in a study of water uptake by bush beans, *Phaseolus vulgaris*. We believe our results on freezing point depressions of complete Hoagland's solutions containing various concentrations of 'Carbowax 4000' may be of value to other investigators.

'Carbowax 4000' was dissolved in Hoagland's complete nutrient solution[2]. The freezing point depression was routinely checked about 24 hr. later. Solutions could be kept a week at room temperature without any effect on freezing-point depression. This lack of ageing effect agrees with the results of Couper and Eley[3]. Beckmann freezing-point apparatus was used for all determinations. For solutions containing 0–60 per cent (w/v) 'Carbowax 4000', a brine solution (−10° C.) was used to lower the temperature; for solutions containing 60–100 per cent (w/v) 'Carbowax 4000', chilled acetone (−20° C.) was used; for solutions containing 100–110 per cent (w/v) 'Carbowax 4000', acetone-dry ice (held at −30° C.) was used. The overlap between freezing solutions was used to maintain continuity.

No difference could be detected in freezing-point depressions of solutions determined in two different freezing mixtures. The results are presented in Fig. 1. It can be seen that the curve, while smooth, deviates from van't Hoff's law. The points on this curve are the result of a single run. The complete sequence was determined several times, however. The greatest deviation from the results presented here was 0·2 deg. C. From results presented here osmotic concentrations can readily be calculated. Couper and Eley[3] give results for freezing-point depressions of 'Carbowax 4000' in water.

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Some New Complexes of Rhenium

No complexes with oxygen or acetylenic ligands of rhenium have been previously reported, and the only known olefin complex was prepared indirectly[1]. We have prepared acetylacetone (rhenium acetylacetonates containing other ligands have been prepared, Nyholm, R. S., personal communication), acetylene and olefin complexes by direct reaction of the ligands with various rhenium compounds.

Acetylacetone reacts on refluxing with rhenium sesquioxide or rhenium dioxide to give rhenium *tris*(acetylacetonate) (Found: C, 36·7; H, 3·7; O, 20·1; Re, 38·9 per cent; required for $Re(C_{15}O_6N_{21})$: C, 36·0; H, 4·3; O, 19·7; Re, 38·3 per cent). The complex forms brown paramagnetic crystals soluble in alcohol, acetone and chloroform. Its infra-red spectrum shows peaks typical of other transitional metal acetylacetonates[2]. Susceptibility measurements show that the effective magnetic moment is dependent on temperature, varying between 1·9 and 2·3 B.M. The variation is probably due to antiferromagnetic effects and the low moment is similar to that of other octahedral Re(III) complexes[3] which have been interpreted by Kotani theory[4].

Very stable monomeric, diamagnetic acetylene complexes were obtained directly from rhenium trichloride and triphenylphosphine rhenium trichloride[5], for example, phenylacetylene gave $ReCl(C_6H_5C{\equiv}CH)_2$ with the trichloride. In these compounds the triple C≡C bond is reduced effectively to a double C=C bond as shown by the shift of the C—C stretching frequency down to 1,700 cm.$^{-1}$, and the acetylene may be regarded as bonded to the metal by two σ bonds from the carbon atoms adjacent to the multiple bond, as suggested by Chatt for the platinum acetylene complexes[6].

Complexes have also been obtained from hydroxy acetylenes; thus, with but-1-yne-3-methyl-3-ol a compound of formula $ReCl(C_5H_8O)_2$ is obtained from the trichloride and $ReCl.P(C_6H_5)_3.(C_5H_8O)_2$ from triphenylphosphine rhenium trichloride. Again the bonding in these compounds is similar to that in the analogous platinum complexes in which it is thought that the acetylene acts as a bidentate ligand with bonding from both the triple bond (μ bond) and the hydroxyl oxygen[7]. The infra-red spectra of the rhenium complexes show similar shifts in both the C≡C and O—H stretching frequencies to those reported for the platinum compounds[7].

Rhenium trichloride and triphenylphosphine rhenium trichloride react with olefins; thus di*cyclo*pentadiene gives $ReCl_2(C_{10}H_{12})_2$ with rhenium trichloride. This black compound is insoluble in all solvents and is probably polymeric. From triphenylphosphine rhenium trichloride a compound $ReCl[P(C_6H_5)_3](C_{10}H_{12})_2$ is obtained which is readily soluble in organic solvents.

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Infra-Red Spectrum of Garnets

Regularities in the infra-red absorption spectra of silicate minerals have been pointed out by Launer[1]. Very recently, a much more detailed study of the strong absorption of the garnets in the 800–1,000 cm.$^{-1}$ range has been published by Wickersheim *et al.*[2]. My results in this field extend down to 280 cm.$^{-1}$ and may be summarized as follows.

(1) If titaniferous garnets are excepted, the general infra-red pattern is pretty uniform, irrespective of the nature of the trivalent and bivalent cations. This pattern is highly characteristic of the garnets, and has not been observed in more than a hundred other silicates so far investigated[3].

(2) The nearly linear relationship between the frequency ν of a given band and the length of the cell edge a_0, mentioned by Wickersheim[2] is verified for all the bands down to 500 cm.$^{-1}$; it does not hold, however, for the bands observed at low frequencies. This is readily explained by the fact that most of these low-frequency bands are related to metal-oxygen vibrations, their position being chiefly determined by the nature of the cation itself.

On the other hand, it should be pointed out that, for certain bands at least, the slope of the straight line ν versus a_0 is slightly but definitely different for the two principal families of garnets, namely, the pyralspite and the ugrandite series.

(3) Garnets of the pyralspite family always exhibit one or two bands of low or medium intensity in the 350–280 cm.$^{-1}$ range. These bands are either missing or extremely weak for the garnets of the ugrandite family. This is reasonably explained if these bands are assigned to metal-oxygen vibrations of the bivalent cation (Fe II, Mn II, Mg) in eight-fold co-ordination: in the ugrandite garnets, this cation is mainly calcium, and the ionic bonds Ca—O should not give rise to discrete infra-red absorption bands.

(4) Some details may be characteristic of a given type of garnet. The discussion of such details is out of place here, and will be given in a forthcoming paper.

(5) In titaniferous garnets of the melanite–schorlomite–ivaarite series, the general infra-red pattern is more or less altered, the degree of alteration being roughly proportional to the amount of titanium. In these garnets, titanium is in four-fold co-ordination (at least partly), and thus replaces silicon. This assignment has been discussed at length in a previous paper[4].

From a more theoretical point of view, the most important feature is the uniformity of the bands assigned to SiO_4 fundamental frequencies. This uniformity is somewhat surprising, as $R^{III}O_6$ octahedra and $R^{II}O_8$ dodecahedra are the nearest neighbours of the SiO_4 tetrahedra, and partial isomorphic replacement of either R^{III} or R^{II} (which always occurs in garnets) should alter the local symmetry around the SiO_4 group.

This is in contrast with the strong influence of the introduction of TiO_4 tetrahedra in the structure. This introduction does alter the infra-red pattern of the SiO_4 tetrahedra, although the TiO_4 and SiO_4 are not mutually linked.

This interesting behaviour is not restricted to the garnet type, and has been observed in other series of isomorphic orthosilicates[5].

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BIOCHEMISTRY

Vitamin A_1 Aldehyde in Hen's Eggs

We have recently reported[1] that vitamin A_1 aldehyde occurs in the ripe eggs of several marine teleost fishes, and is the predominant form of vitamin A in them. In that paper we stated that we could not detect vitamin A_1 aldehyde in hen's eggs, but further experiments have shown it to be present.

Eggs from inseminated Light Sussex hens on a breeder's mash containing 2·4 mgm./kgm. of pre-formed vitamin A were used, the eggs being stored at 4° C. until used. The yolk was separated from the white, and extracted as previously described for fish eggs[1]. When the extract was subjected to gradient elution chromatography[1], with a large column to take 2 gm. of lipid, the fractions that might be expected to contain vitamin A_1 aldehyde gave in the Carr–Price test only a fleeting blue colour, which soon became reddish-brown. Ultra-violet absorption curves gave no definite proof of the presence of aldehyde. However, when these fractions were reduced with sodium borohydride and the product subjected to chromatography by a modification[2] of the method of Thompson, Ganguly and Kon[3], vitamin A_1 alcohol was found by the Carr–Price test to be present, and was presumably derived from vitamin A_1 aldehyde in the original extract, the calculated amount being about 11 μgm. in the whole yolk. The other lipid material in the fractions apparently interfered with the direct characterization of the aldehyde.

In herring eggs, the vitamin A_1 aldehyde is bound to a lipid and, either directly or indirectly, to protein[1]. If it were so in the yolk of hen's eggs, then by first extracting the lipids with a fat solvent without denaturing the proteins, and then denaturing them and extracting again, it might be possible to separate the aldehyde from most of the lipid. To test this possibility, each of several yolks was freeze-dried, ground to a powder and extracted with diethyl ether

in a Soxhlet apparatus for 4 hr. The residue was then treated with 30 ml. water and 60 ml. ethanol and extracted with three 200 ml. lots of light petroleum in a Waring 'Blendor' under nitrogen. The two extracts were taken to dryness and the residues weighed, dissolved in *n*-hexane and vitamin A_1 esters and alcohol[2,3] and vitamin A_1 aldehyde[1] determined. The diethyl ether extract contained 75–80 per cent of the total lipids, 95 per cent of the total free and esterified vitamin A_1, but no vitamin A_1 aldehyde (qualitative test by reduction to the alcohol form). The light petroleum extract contained 20–25 per cent of the total lipid, 5 per cent or less of the total free and esterified vitamin A_1 and all the vitamin A_1 aldehyde, in amounts of about 20 μgm./egg. This aldehyde was identified by its chromatographic behaviour, by its characteristic absorption maxima in the ultra-violet and in the Carr–Price test, and by formation of vitamin A_1 alcohol under the action of sodium borohydride. No vitamin A_2 aldehyde was detected.

Table 1. Mean Values for Weights of Whole Egg and of Yolk and for Total Amounts of Vitamin A_1 Esters, Alcohol and Aldehyde in Eggs from two Light Sussex Hens

Bird	No. of eggs	Weight of whole egg (gm.)	Weight of yolk (gm.)	Vitamin A_1 esters* (μgm.)	Vitamin A_1 alcohol (μgm.)	Vitamin A_1 aldehyde (μgm.)
A	4	60·1	19·5	17·7	90	21·8
B	3	59·2	19·1	19·8	109	22·4

* Expressed as the equivalent quantity of vitamin A_1 alcohol.

No vitamin A in any form was found in egg white, which contains a negligible quantity of lipid, so that measurements on the yolk apply to the whole egg. Table 1 shows that, as has been previously reported[4], vitamin A_1 alcohol is the predominant form in hen's eggs, and that vitamin A_1 esters and vitamin A_1 aldehyde are present in about equal amounts.

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Effect of Diphenylamine on the Synthesis of Carotene

A number of authors have shown that diphenylamine inhibits the synthesis of carotenoids mainly by micro-organisms[1–4]. We have carried out investigations with a red yeast rich in carotenoids in order to interpret the mechanism of this inhibition.

In 1953, György Németh isolated from root nodules of *Lupinus luteus* certain micro-organisms which produced in a *Rhizobium* nutrient a red pigment and which were capable of fixing atmospheric nitrogen[5–7].

We used the same type of yeast. The micro-organism was allowed to multiply in Roux flasks in the form of a surface culture. The nutrient contained 5 per cent bean extract, and to it was added 5 and 20 mgm. of diphenylamine, respectively, in 100 ml.

On the agar nutrient containing 5 mgm. of diphenylamine, the content of carotenoids of the micro-organism essentially decreased and the red colour faded. On raising the concentration of diphenylamine to 20 mgm., the culture became completely colourless. Moreover, the quantity and nature of the carotenoids produced changes. In the presence of 5 mgm. of diphenylamine the content of carotenoids was 110 μgm. in 10 gm. of culture, and in the presence of 20 mgm. of diphenylamine it was 40 μgm. as compared with 285 μgm. in control cultures[8].

Control cultures free of diphenylamine contained torulin, γ- and β-carotene, and torularhodin. Of these compounds, torulin was present in the greatest quantity. On applying 5 mgm. of diphenylamine, the concentrations of carotenoids decreased by a similar extent, while in the presence of 20 mgm. of diphenylamine, neither torularhodin, the sole hypophasic pigment, nor γ-carotene could be detected. Accordingly, on evaluating the data of distribution between solvents, of chromatograms and of spectra[8], it can be stated that higher concentrations of diphenylamine inhibit the synthesis of the hypophasic pigment torularhodin and of γ-carotene, both of which belong, from a genetical aspect, to the group of 'younger' carotenoids. Obviously also the synthesis of other carotenoids is simultaneously reduced to a similar extent.

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Interaction between Albumin, Lecithin and Cephalin

Lecithins and cephalins form more or less stable combinations with proteins[1,2]. These are believed to be the result of lipid–protein interaction[3]. Provided the method is applicable, information regarding the interaction may be obtained by an electrophoretic method[4] used for the study of interaction between dextrans and proteins. Essentially, this method uses the Antweiler micro-Tiselius apparatus to obtain spikes, at various *p*H's and concentrations, of the two interacting substances and of their mixtures. Plotting the areas under the spikes against concentration enables one to determine a function of α, the specific refractive increment, and sometimes to decide as to whether the mixture of the two components has resulted in interaction between them.

In the case of albumin (Cohn's fraction V) and lecithin dissolved in alcohol or cephalin dissolved in wet ether, the protein undergoes a change (possibly denaturation) at 20° C., the temperature used for electrophoresis. This factor must be dealt with first.

1*a*. *'Denaturation' of albumin by alcohol at 20° C.* To 2 ml. of 4 per cent albumin dissolved in Michaelis buffer at *p*H 6·6 is added 1 ml. of the buffer and 1 ml. of 25 per cent, 15 per cent, and 5 per cent ethanol; after standing at 20° C. for 4 hr., the three systems are dialysed against Michaelis buffer at *p*H 6·6 for 8 hr. at 20° C. Electrophoretic patterns are obtained for each system, ascending migration being allowed to occur for 6 min. The areas under the spikes are measured and compared with the area under the spike (6 min. ascending migration) of a 2 per cent albumin in buffer at *p*H 6·6. For example, the area under the albumin spike was 86 arbitrary units, that under the spike for albumin plus 25 per cent ethanol 56 units, that under the spike for albumin plus 15 per cent ethanol 60 units, and that under the spike for albumin plus 5 per cent ethanol 70 units. Increasing concentration of ethanol reduces the area under the albumin–ethanol spike; the important reduction is that produced by 25 per cent ethanol, which is from 86 to 56 units. Call $56/86 = 0{\cdot}65$ or the factor F.

1*b*. *Effect of ether on albumin at 20° C.* Proceeding in exactly the same way as the above, at *p*H 6·6, and comparing the area under a 2 per cent albumin spike with the area under a 2 per cent albumin spike in a system containing 6·65 per cent of ethyl ether (nearly the highest concentration of ether miscible with water), the effect of the ether is slightly to increase the area under the albumin spike, F being 1·12. This small effect (10 per cent increase) may indicate an effect of ether on albumin, but not one like that produced by ethanol. There is no reason for thinking that what may be a 'denaturation effect' should not decrease the spike area in the case of ethanol, and increase it in the case of ether.

2*a*. *Interaction of albumin with lecithin.* The system contains 2 ml. of 4 per cent albumin in buffer at *p*H 6·6, 1 ml. of the buffer, and 1 ml. of Polanyi's lecithin (that is, about 25 mgm.) in absolute ethanol (Polanyi, M., private communication); the mixing of these components reduces the protein to 2 per cent, and the ethanol to 25 per cent. After standing for 4 hr. at 20° C., the mixture is dialysed against Michaelis buffer at *p*H 6·6 for 8 hr. at 20° C. The electrophoretic pattern now shows a spike moving at about the same rate as albumin moves; dialysed lecithin gives no electrophoretic spike. At 6 min. the area under the spike observed was 64 units, but this figure must be divided by $F = 0{\cdot}65$ to allow for the 'denaturing effect' of 25 per cent ethanol. The division gives 98·5 units instead of 86 units for albumin alone. A reasonable explanation of this is that there has been an albumin–lecithin interaction, a new species having been formed which increases the area under the albumin–lecithin spike from 86 units (for the albumin alone) to 98·5 units for the new species, that is, an increase of about 15 per cent. Other experiments show increases of 14–20 per cent. The refractive index increment for the albumin is 0·0018, so that for the new species would be about 0·00204, assuming that the concentration of the new species is exactly the same as that of the albumin.

2*b*. *Effect of cephalin in ether on albumin at 20° C.* Using a system containing 2 ml. of 4 per cent albumin, 1·75 ml. of Michaelis buffer at *p*H 6·6 and 0·25 ml. of saturated cephalin in ether, electrophoretic patterns were obtained for 2 per cent albumin and for the mixture containing 2 per cent albumin plus cephalin in ether. Comparing the areas under the spikes, the incorporation of cephalin in ether produced substantially the same effect as that of ether alone, that is, an increase under the spike of about 10 per cent. Considering the interaction produced with albumin by lecithin in 25 per cent alcohol, the absence of an effect of cephalin in ether may seem surprising, but it has to be remembered that solubility determines that the quantity of cephalin in the protein–cephalin–ether systems is only 2·5 mgm., while the amount of lecithin in the protein–lecithin–ethanol systems is about 25 mgm. The latter gave a 15 per cent increase in spike area by interaction; the former, if the effect were proportional to weight, would give only a 1·5 per cent increase, which could be missed.

Finally, if washed red cells are added to the dialysed protein–lecithin–ethanol system, examination between plastic surfaces shows that they are spheres, that is, there is enough lecithin in the system to sphere them in spite of the albumin. If washed red cells are added to the dialysed protein–cephalin–ether system, they remain crenated disks, presumably because the concentration of cephalin is too small to render them spherical.

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Smilagenone and *Epi*-Smilagenin from *Dioscorea* Saponin

In examining the effect of prolonged keeping of *Dioscorea* yams[1] in contact with water, fresh yams of *Dioscorea prazeri* and *D. deltoidea*, after being cut to small pieces and crushed, were mixed with sufficient water for different periods ranging from a few weeks to several months. The filtered residue was dried and extracted with petroleum ether (b.p. 40–60°) in a Soxhlet.

From the petroleum ether extract a colourless crystalline product was obtained. On chromatography over alumina it yielded mainly the following products: (*A*) $C_{27}H_{42}O_3$, stout needles, m.p. 188–89°; $[\alpha]_D^{21} - 52°$ ($CHCl_3$) and (*B*) $C_{27}H_{44}O_3$, fine silky needles, m.p. 220–22°; $[\alpha]_D^{21} -43{\cdot}8°$ ($CHCl_3$). Product *A* failed to give any acetyl derivative, but formed a semicarbazone, $C_{28}H_{45}O_3N_3$, m.p. 222–23° (decomp.). On catalytic hydrogenation in acetic acid it yielded product (*B*), which again on oxidation with chromic acid gave back product (*A*). Product (*B*) formed a monoacetate, $C_{29}H_{46}O_4$, m.p.

163–64°, and was identified as *epi*-smilagenin by a comparison of the mixed melting point with an authentic specimen. Product (*A*) was similarly identified as smilagenone.

In this connexion it may be mentioned that Marker *et al.*[3] showed that dehydrotigogenone (diosgenone) when administered to a dog on biscuit diet gave diosgenin, smilagenin and *epi*-smilagenin.

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Thiobarbituric Acid Spray Reagent for Deoxy Sugars and Sialic Acids

Recently, new sensitive assays have been reported for deoxy sugars[1], 2-keto,3-deoxy sugar acids[2–4], and sialic acids[5,6]. In these assays, the products of periodate oxidation, malonaldehyde from deoxy sugars and β-formylpyruvic acid from the latter two groups of compounds, are coupled with 2-thiobarbituric acid to produce a bright red chromophore. I wish to report an adaptation of these methods for spraying paper chromatograms.

The procedure for the detection of deoxy sugars and 2-keto,3-deoxygluconic acid is as follows. After removal of solvent the paper is sprayed with a 0·02 *M* aqueous solution of sodium periodate. After 15 min. a solution consisting of ethylene glycol, acetone and concentrated sulphuric acid (50 : 50 : 0·3) is sprayed on to the paper. After 10 min. the paper is sprayed with an aqueous solution of 6 per cent sodium 2-thiobarbiturate. (Although several chemical supply companies sell '2-thiobarbituric acid', we have found that the product is frequently mislabelled and that the product is a salt of 2-thiobarbituric acid. Only 0·9 gm. of the acid is soluble in 100 ml. water, whereas 6·5 gm. of the sodium salt is soluble in the same amount of water. The free acid may be rendered soluble by the addition of an equimolar amount of sodium hydroxide.) Red spots appear after heating at 100° for 5 min. Under an ultra-violet light ('Mineralight') the spots give a red fluorescence. As little as 0·5 μgm. of 2-deoxyribose can be seen. We have used the spray to detect 2-deoxyglucose, 2-deoxyxylose, and 2-deoxygalactose. Although 3-deoxyglucose can also be detected on paper, the colour intensity of the spot is considerably less than that of an equimolar amount of 2-deoxyglucose.

For the detection of sialic acids a solution of 0·05 *M* sodium periodate in 0·05 *N* sulphuric acid is used. The glycol and thiobarbituric acid solutions are the same as above. A longer heating time of about 10 min. is required. Approximately 3 μgm. of sialic acids can be detected.

Using this procedure, deoxy sugars and sialic acids have been successfully located on Whatman No. 1, 3 and 3 *MM* paper, using a variety of acidic, basic and neutral solvent systems.

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Enzymatic Deamination of 5-Deoxycytidylic Acid and of 5-Methyl-5′-deoxycytidylic Acid in Growing and in Non-growing Tissues

The deamination by enzymatic hydrolysis of 5′-deoxycytidylic acid to 5′-deoxyuridylic acid and of CH_3-5′-deoxycytidylic acid to 5′-deoxythymidylic acid has been previously reported in sea urchin egg homogenates[1,2], and in adult rabbit liver[3].

The deamination of 5′-deoxycytidylic acid and of CH_3-5′-deoxycytidylic acid has been investigated in the developing sea urchin embryo[4], in normal and regenerating rabbit liver, in adult rats and in embryonic rat tissues, in adult chick and in chick embryos in order to correlate these enzyme activities with biosynthesis of deoxyribonucleic acid and hence with the metabolic activities of tissues that grow by increase in the number of the component cells.

In this preliminary communication some of our results for tissues of warm-blooded animals are reported. These clearly show that the 5′-deoxycytidylic acid and the CH_3-5′-deoxycytidylic acid deaminations occur to a greater extent in growing tissues than in normal non-growing tissues. In adult tissues of rat and of chick it is impossible to detect with our assay the activities in the unfractionated homogenate.

The tissues, as soon as they were removed from the animal, were homogenized in a Potter–Elvehjem glass homogenizer with phosphate buffer. The homogenate was centrifuged for 15 min. at 4° C. and at 18,000*g*. The supernatant was used for the enzymatic assay.

The enzymatic assays are based on the ultra-violet spectral variations caused by the deamination. Tables 1 and 2 show the assays. All the enzymatic determinations have been made in the range in which

Table 1. Assay of 5′-Deoxycytidylic Acid Deaminase

Time (min.)	Absorbance at : 232 mμ	250 mμ	267 mμ	280 mμ
0·5	0·120	0·175	0·440	0·530
6	0·120	0·195	0·445	0·495
11	0·115	0·215	0·440	0·450
Δ*A*	−0·005	+0·040	0	−0·080

Centrifuged supernatant of a homogenate from chick embryo's liver : protein, 2 mgm. ; 5′-deoxycytidylic acid, 1 μmole ; *tris*, 30 μmoles. Final volume, 0·5 ml. ; *p*H 7·2. Incubation at 38° C. At the indicated times 0·1 ml. was removed and 0·1 ml. 10 per cent perchloric acid was added. After high-speed centrifugation 0·1 ml. of the clear colourless supernatant was diluted with 2·4 ml. of 0·01 *N* hydrochloric acid, and the absorbance of this solution was determined with a Beckman *DU* spectrophotometer.

Table 2. Assay of CH_3-5′-Deoxycytidylic Acid Deaminase

Time (min.)	Absorbance at : 236 mμ	260 mμ	273 mμ	290 mμ
0·5	0·175	0·345	0·545	0·560
6	0·175	0·360	0·540	0·525
11	0·170	0·375	0·545	0·490
ΔA	−0·005	+0·030	0	−0·070

The conditions were those of Table 1, except for the substitution of 5′-deoxycytidylic acid by 1 μmole of CH_3-5′-deoxycytidylic acid.

Table 3. Relative Specific Activities of 5′-Deoxycytidylicase and CH_3-5′-Deoxycytidylicase in Resting and Growing Tissues

Animal	5′-Deoxy-cytidylicase	CH_3-5′-Deoxy-cytidylicase
Chick embryos' liver	40	35
Adult chick liver	0	0
Rat embryos without liver	34	29
Rat embryos' liver	30	18
Adult rat liver	0	0
Adult rabbit liver	17	13
Adult rabbit liver in regeneration after partial hepatectomy	28	24

The conditions of the assays were those of Tables 1 and 2. The specific activities of the two enzymes are measured respectively as ΔA 280 mμ and ΔA 290 mμ, multiplied by 10^3 per 10 min. per mgm. of protein.

there is direct proportionality of the spectral variations with the amount of enzyme used.

When, instead of the deoxynucleotides, the corresponding deoxynucleosides or bases are used as substrates in the conditions of our assay, the absence of spectral variations in embryonic tissues of chick and of rat, and the absence of significant spectral variations in rabbit liver, indicate that the direct deamination of 5′-deoxycytidylic acid and of CH_3-5′-deoxycytidylic acid is being measured.

Furthermore, in every case 5′-deoxyuridylic acid and 5′-deoxythymidylic acid have been isolated in good yield from incubation mixtures containing ten times the amounts indicated in Tables 1 and 2. The techniques used for the isolation have been described in refs. 1 and 2.

The results presented in this communication, and other results on the behaviour of 5′-deoxycytidylic acid and CH_3-5′-deoxycytidylic acid deamination in the developing sea urchin embryo[4], emphasize the importance of these enzyme activities in biosynthesis of deoxyribonucleic acid and in cellular multiplication.

These results will be reported in detail elsewhere.

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A Methyl Cytidine from the Ribonucleic Acid of *Anacystis nidulans*

A project was undertaken to characterize the nucleic acids from *Anacystis nidulans*, a blue-green alga. The present communication deals with the characteristics of ribonucleic acid and a new cytidine compound from it.

Freshly harvested cells grown in a continuous culture chamber[1] were used for isolating ribonucleic acid from *Anacystis nidulans*. It was found that the method of Ogur and Rosen[2] was most suitable. Ribonucleic acid constitutes 0·37–0·50 per cent of the fresh weight of the cells. Ribonucleic acid thus isolated was purified from protein with octanol–chloroform mixture and from polysaccharides with 2-methoxy ethanol[3]. The percentage of moles of different bases and nucleotides were estimated by ascending paper chromatography[4] and electrophoresis[5]. These results, together with nitrogen and phosphorus ratio, are given in Table 1. Ribonucleic acid records a maximum absorption at 260 mμ and minimum at 230 mμ. It shows a preponderance of guanylic acid compared to other nucleotides.

Table 1. Percentage of Moles of Nucleotides in Ribonucleic Acid

Extraction	N (per cent)	P (per cent)	N/P	Max. (mμ)	Min. (mμ)	GA	AA	CA	UA
						Mole (per cent)			
10 per cent NaCl*	14·80	6·45	2·3	260	230	36	25	20	19
$HClO_4$	15·57	8·25	1·89	260	230	39	22	20	19

* Extraction of ribonucleic acid with 10 per cent sodium chloride was done according to the procedure of Heinrich *et al.* (ref. 8).

After hydrolysing ribonucleic acid with hydrochloric acid for 1 hr. at 100° C. the cytidylic acid was recovered from the paper chromatogram[4]. The cytidylic acid thus isolated was incubated with alkaline phosphatase (purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio) in 0·2 *M tris* buffer at *p*H 9·0. After standing for 4 hr. at room temperature the incubated mixture was chromatographed[4]. It was observed that cytidylic acid was fully converted to cytidine within that time when the concentration of enzyme was 8–10 per cent of the substrate weight. The cytidine was further subjected to electrophoresis in borate buffer *p*H 9·2 for 6 hr. at a voltage gradient of 11 V./cm. The cytidine was resolved into two spots and the mobilities of those compounds are recorded in Table 2. The spots were eluted with 0·1 *N* hydrochloric acid and the spectra were taken on a recording Cary spectrophotometer. The spectral characteristics of the new compound, which resembles mostly cytidine, is given in Fig. 1. The sugar moiety of cytidine compound was further analysed after hydrolysis with 0·05 *N* sulphuric acid and the characteristic mobility of the sugar in borate buffer is given in Table 2. From the characteristics of the sugar moiety, cytidine from *Anacystis nidulans* contains 2-O-methyl ribose. The optical density at 280 mμ of methyl cytidine varies from 0·2 to 0·25 per cent of the total optical density at 280 mμ. Methyl ribosides of purines and pyrimidines have recently

Table 2. Electrophoretic Mobility of Cytidine Compounds and Pentoses

Substances	Mobility	Substances	Mobility
Deoxycytidine	0·61	Sugar from *X*-cytidine	0·57
X-cytidine (unknown)	0·72	2-O-methyl ribose	0·60
Cytidine	1·00	3-O-methyl ribose	0·83
Deoxyribose	0·48	Ribose	1·00

Electrophoresis was carried out in borate buffer *p*H 9·2 for 6 hr., voltage gradient being 11 V./cm. The movements of the compounds are towards anode.

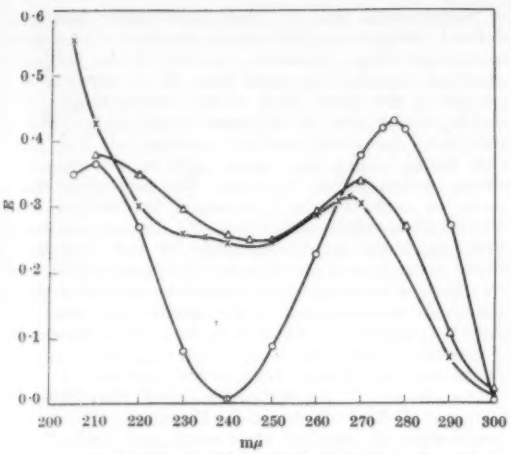

Fig. 1. Ultra-violet absorption spectrum of *X*-cytidine from ribonucleic acid. ○, *p*H 2·0; ×, *p*H 7·2; △, *p*H 11·5

been reported in some ribonucleic acid by Smith and Dunn[6]. (Methylated trityl adenosine was generously supplied by Dr. J. D. Smith from which 2-O-methyl and 3-O-methyl ribose were separated according to Brown *et al.*[7].) Other methyl ribosides were not detected in our preparations.

The precise role of this type of cytidine or other nucleosides or nucleotides in any type of ribonucleic acid is unknown. However, it may not be out of order to speculate upon their involvement in specific protein synthesis.

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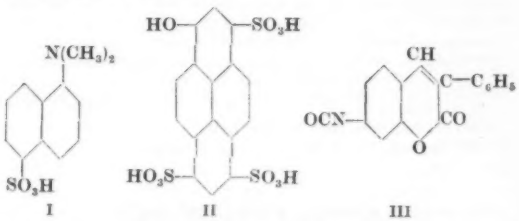
Depolarization of the Fluorescence of Proteins labelled with Various Fluorescent Dyes

The technique by which measurements of the depolarization of fluorescence of protein conjugates is used for investigating rotational relaxation has been applied mainly with the fluorescent dye, 1-dimethylamino-naphthalene-5-sulphonic acid (I) [1,2]. The life-time of the excited state (τ) of this dye when conjugated to protein has been measured by independent methods by Weber[3] and by Steiner and McAlister[4]. Good agreement was reported, and in the present work the value $\tau = 1{\cdot}18 \times 10^{-8}$ sec. (probably accurate to $\pm 0{\cdot}05 \times 10^{-8}$), the mean of several determinations on conjugates with bovine serum albumin by Steiner and McAlister, has been assumed throughout. Assuming an absolute error in the experimental estimates of degree of polarization (p) of $\pm 0{\cdot}003$, it can be shown that the dye (with $p_0 = 0{\cdot}25$, the subscript referring to zero value of the temperature/viscosity ratio) is adequate for measuring relaxation times in the range $0{\cdot}5$–30×10^{-8} sec. to an accuracy of $\pm$ 10 per cent. These relaxation times correspond to a molecular weight range of 5,000–300,000 if the molecule is spherical and unhydrated. The corresponding molecular weights for hydrated and moderately asymmetric molecules will be less, usually by a factor of at least 2 or 3. The range is determined mainly by the magnitude of τ and the accuracy of measurements of the degree of polarization of the fluorescent light. If dyes having longer and shorter values of τ were available, the range of the method would be considerably extended.



The present communication describes the use of two such dyes: 3-hydroxypyrene-5:8:10 trisulphonic acid (II) with a longer life-time, and 3-phenyl-7-*iso*cyanato-coumarin (III) with a shorter life-time. Both dyes conjugate easily with proteins[5,6]. Such conjugates with bovine serum albumin have been prepared, and the τ values in each conjugate determined by Weber's method[3]. The degree of polarization of fluorescence of the conjugate was measured through a yellow filter (Ilford 110) over a range of temperature. Plotting the reciprocal of the degree of polarization of fluorescence as a function of the temperature/viscosity ratio, it was possible to calculate the τ values using Perrin's modified equation[3] and assuming Oncley's values for the principal relaxation time of bovine serum albumin[7]. From these results the range of relaxation times (ρ) measurable to $\pm$ 10 per cent with each type of conjugate has been calculated together with the corresponding range of anhydrous spherical molecular weights; these results are shown in Table 1. It can be seen that the pyrene derivative has a life-time some eight times greater, and the coumarin derivative almost five times smaller than the naphthalene compound. The range of relaxation time (and molecular weight) which can be investigated by this technique is correspondingly extended.

The total range of molecular weights covered by conjugates prepared from these three dyes is 1,000–2,500,000 for spherical unhydrated molecules, or

Table 1. Range of the Depolarization Method

Dye	$\tau \times 10^{-8}$ sec.	Range of relaxation times $\times 10^{8}$	Molec. weight range $\times 10^{-3}$ (spherical molecules)
3-Hydroxypyrene 5 : 8 : 10 trisulphonic acid	9·0	4–250	40–2,500
1-Dimethylamino-naphthalene 5-sulphonic acid	1·18	0·5–30	5–300
3-Phenyl-7-*iso*-cyanato-coumarin	0·25	0·1–7	1–60

about 400–1,000,000 for typical, moderately asymmetrical molecules. Experiments have already shown the usefulness of the newer dyes with certain protein molecules which are either too large or too small for investigation with the naphthalene dye[6], but it would be rash to expect that all such cases would be successful. The occurrence of protein inactivation, of specific protein effects on the fluorescent life-time, the onset of intramolecular rotations, and similar effects are largely unpredictable and must be excluded before a useful application in any one case can be proved.

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ANIMAL PHYSIOLOGY

Urinary Phenols in Stress

Abnormally high rates of excretion of 4-hydroxy-3-methoxymandelic acid, a metabolite of both adrenaline and noradrenaline, are now well known to be associated with phaeochromocytoma and sympathoblastoma[1–3]. A convenient chromatographic technique enabling the substance to be estimated visually has been described elsewhere[3]. It seemed of interest to examine the possibility that the method would prove sufficiently sensitive to detect the increased secretions of the catecholamines to be anticipated in subjects exposed to stress.

In the first place we have examined urines from hospital patients under severe conditions of stress occasioned by spinal shock, surgical operations, accident injuries and burns. In contrast to young normal males who excreted 4-hydroxy-3-methoxymandelic acid at average rates varying from 1 to 2·5 μgm./min. (values not corrected for incomplete recovery from urine), many of these subjects excreted the acid in much greater quantity (4–10 μgm./min.). The behaviour of patients having complete transverse lesions of the cervical cord has proved of particular interest, since in these subjects the whole efferent sympathetic outflow is isolated from the control of the higher nervous centres. The excretion-rate of 4-hydroxy-3-methoxymandelic acid was observed to be markedly subnormal in fit subjects of this type, but was within the normal range in those under conditions of stress; this increase in stress may possibly be due to adrenaline release by a direct action of histamine on the adrenal medulla.

More detailed chromatography has indicated that other phenols may be excreted in increased amounts in stress. The most noticeable changes in the present experiments occurred with *p*-hydroxyphenyllactic acid; in spinal shock at least, the appearance of this substance is probably related to the difficulty of saturating the patients with ascorbic acid. Increased excretion of *p*-hydroxy-phenylpyruvic acid also occurred in some subjects. Increases in the excretion of homovanillic acid in stress were rather less well defined owing to the invariable presence of this acid in normal urine; however, several of the subjects excreted considerably more than the 8 mgm./24 hr. quoted as the upper limit of the normal range[4]. It seems possible that an increased metabolism of 3 : 4-dihydroxyphenylethylamine is implicated, and that this amine might play some part in the response of the human subject to stress. The two urines from cases of neuroblastoma examined by Sandler and Ruthven[2], to whom we are grateful for samples, have been examined chromatographically and both were found to contain large amounts of homovanillic acid in addition to 4-hydroxy-3-methoxymandelic acid; abnormal excretion of homovanillic acid was not noted in eight cases of phaeochromocytoma, however. A further example where excretion was high occurred in the one case of argentaffinoma examined.

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Postural Changes in Chlorpromazine Hypothermia

The relatively small disturbance in the chemical component of thermoregulation in the rat[1] after the injection of chlorpromazine cannot explain completely the expressive hypothermia observed with this drug[2]. LeBlanc presumes that the physical component of thermoregulation as the result of postural changes caused by chlorpromazine[3,4] plays a significant part. In work presented in this communication, I have aimed at a closer definition of these postural changes and further at determining their relation to the changes of body temperature.

Male albino rats weighing 180 gm. were used. Chlorpromazine (1 per cent) was injected intraperitoneally at a dosage of 20 mgm./kgm. The room temperature was maintained at 20 ± 1° C. Body temperature was measured by rectal insertion of a thermocouple to a depth of 30 mm. The postural placing reactions and righting reflexes were determined[5] at various time-intervals after the administration of chlorpromazine and their presence or absence in the group of animals was expressed in percentages.

Table 1 shows that the courses of the extinction of placing reactions and righting reflexes differ from each other. Whereas the cortical placing reactions disappear in nearly all the animals only after a significant drop of body temperature (in 15 min.) and are absent for whole hours, the righting reflexes of the brain stem start to disappear earlier, but return gradually later on. It follows that the cylindrically shaped posture of chlorpromazine-treated rats, with their extremities spread away from the body, is

Table 1. Relation between Body Temperature and Changes of Posture after Chlorpromazine

	n	Time after injection of chlorpromazine (min.)						
		0	3	6	15	90	180	390
Body temperature (° C.)*	40	37·3 ± 0·5	37·2 ± 0·5	36·5 ± 0·4	34·9 ± 0·4	31·7 ± 0·4	29·2 ± 0·3	31·3 ± 0·3
Placing reactions†	43	0·0	0·0	13·8	89·4	78·5	84·7	90·4
Righting reflexes†	43	0·0	42·8	73·3	57·4	52·6	43·5	37·8

* Mean ± *S.E.*
† Percentage of animals in the group where they are absent.

induced mainly by the disturbance of placing reactions. The reversible exclusion of these reactions is mainly responsible for the exposure of a wider surface of the animal's body to a lower temperature of the environment at the time when the chemical component of thermoregulation decreases.

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Release of Acetylcholine in Rat Diaphragm

Several attempts have been made in the past to correlate the amount of acetylcholine released during activity at nerve endings with the amount needed to produce activation of the effector cell[1–3]. It is clearly of crucial importance for the acetylcholine hypothesis that there should be quantitative agreement between these amounts. Until recently, a very substantial discrepancy has remained, supporting the suggestion that acetylcholine is merely a by-product of transmission. One source of difficulty in making such correlations has been the paucity of results concerning the release of acetylcholine and the excitation produced by its application in the same preparation.

Intracellular recording of end-plate activity in muscle, and the ionophoretic application of acetylcholine in controlled amounts from micro-pipettes, have made possible a detailed analysis of end-plate potentials under conditions closely approaching those which obtain during normal transmission at the neuro-muscular junction. End-plate potentials have been produced by such methods in the isolated rat diaphragm with as little as $1{\cdot}5 \times 10^{-17}$ mole of acetylcholine[4]. We have now found that acetylcholine is released by nerve endings in the rat diaphragm, during stimulation of the phrenic nerve, in amounts which are of the same order of magnitude.

The isolated phrenic nerve diaphragm preparation was stimulated in a 'Perspex' chamber at room temperature (19–23° C.). The muscle was bathed in 5·0 ml. of Ringer–Locke solution continually agitated by oxygen bubbles, and the rate and duration of nerve stimulation were kept to a minimum consistent with the sensitivity of the preparation used for assay (the leech dorsal muscle, the heart of *Mya areneria* or the rat duodenum). To prevent destruction of acetylcholine, we either added physostigmine sulphate to the solution (10^{-5}), or administered di*iso*propyl fluorophosphonate to the rat (1 mgm./100 gm. body-weight). For control, active solutions were treated with strong alkali whenever possible.

It is difficult to be certain in this type of experiment that one is exciting all the nerve endings. The phrenic nerve was always stimulated with shocks two or three times greater than maximal, and the action potential of the nerve trunk was recorded close to its entrance into the muscle throughout the period of stimulation. This gave us a useful index of failure of excitation or conduction in the nerve, but no positive information about the activation of the nerve endings.

During periods of stimulation at a rate not greater than 5/sec. and lasting not more than 5 min., the amounts of acetylcholine collected from hemi-diaphragms varied between 0·4 and $2{\cdot}5 \times 10^{-13}$ mole per impulse. Fourteen estimates gave a mean value of $1{\cdot}1 \times 10^{-13}$ mole. Since there are about 10,000 muscle fibres in the hemi-diaphragm[5], the mean release per ending was $1{\cdot}1 \times 10^{-17}$ mole.

It is clear that in the rat diaphragm there is overlap between the least amount of acetylcholine needed to produce appreciable excitation at an end-plate ($1{\cdot}5 \times 10^{-17}$ mole) and the greatest amount released at nerve endings during stimulation ($2{\cdot}5 \times 10^{-17}$ mole). The latter should be more significant than the mean value, since the principal sources of error, imperfect activation of nerve endings and hydrolysis of acetylcholine, would both lead to an underestimate.

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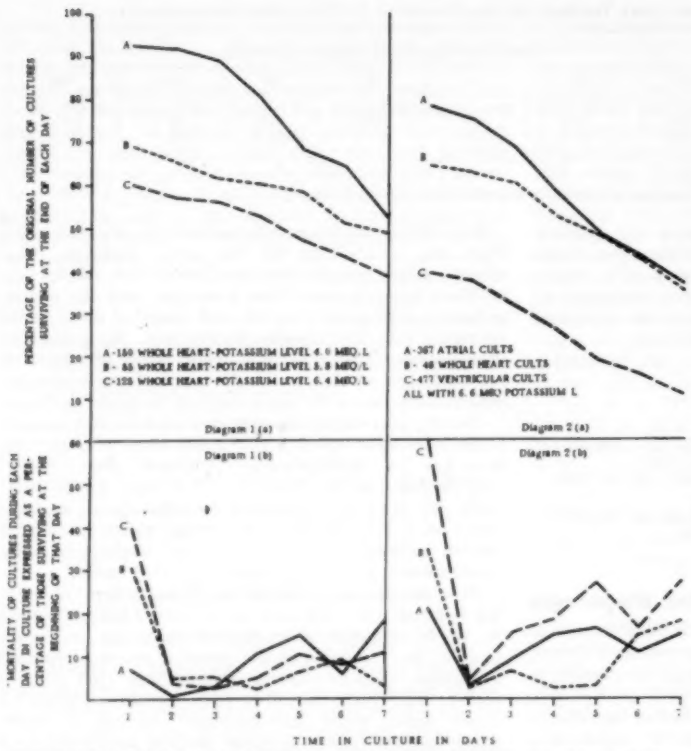
Effect of Supernatant Potassium-Level on Cardiac Activity in Quantitative Tissue Culture

Inadequate functional 'survival' of hearts cultured in supernatant containing serum with a high potassium-level from lysed red cells led us to investigate the effects of supernatant potassium-levels on the electrical activity of 1,222 hearts in quantitative tissue culture. The criterion for 'adequate survival' is an ability to produce potentials of at least 0·02 mV. during a minimum of three periods of observation per day (8 a.m., 1 p.m. and 5 p.m.). The apparatus, etc., is described in previous papers[1]. The supernatants are 0·25 per cent solutions of human serum protein in balanced salt solutions—however, they differ in potassium-level. The 'adequate survival' time is given to the nearest day using the smallest figure in cases of doubt.

Fig. 1*a* relates time and percentage adequate 'survival' for three separate series of 6-day chick embryo whole hearts, all from the same breeder.


PERCENTAGE OF THE ORIGINAL NUMBER OF CULTURES SURVIVING AT THE END OF EACH DAY
A-150 WHOLE HEART-POTASSIUM LEVEL 4.0 MEQ./L
B-85 WHOLE HEART-POTASSIUM LEVEL 5.8 MEQ./L
C-125 WHOLE HEART-POTASSIUM LEVEL 6.4 MEQ./L
A-367 ATRIAL CULTS
B-48 WHOLE HEART CULTS
C-477 VENTRICULAR CULTS
ALL WITH 6.6 MEQ POTASSIUM/L
Diagram 1 (a)
Diagram 2 (a)
Diagram 1 (b)
Diagram 2 (b)
MORTALITY OF CULTURES DURING EACH DAY IN CULTURE EXPRESSED AS A PERCENTAGE OF THOSE SURVIVING AT THE BEGINNING OF THAT DAY
TIME IN CULTURE IN DAYS

Figs. 1 and 2. The electrical functional survival and mortality of cultures of 6-day chick embryo whole hearts, isolated right atria and isolated right ventricles in supernatants of various potassium-levels

These series are grown in supernatants of different potassium-level and those in the supernatant of lowest potassium-level show the best 'survival'. The considerable initial 'survival' difference between the three series (particularly between series *A* and *B*) lessens with time. Supernatants *B* and *C* are made from the same serum containing lysed red cells but, since a low-potassium balanced salt solution is used to make supernatant *B*, 'hey differ only in potassium-level. Thus, *B*'s better 'survival' makes it probable that, of lysed red cell products, the potassium has the greatest effect on 'survival' of hearts in culture. The small initial 'survival' in high potassium supernatants may be due to release of potassium from damaged muscle raising the total potassium-level around nearby healthy muscle fibres to the critical level which interferes with their electrical functions.

Fig. 1*b* shows the relationship between time and the number of hearts failing to 'survive' at the end of each day expressed as a percentage of the number of hearts surviving at the beginning of the same day. The first day's high 'mortality' supports the theory of the damaging effect of high local potassium-levels (a combination of high supernatant potassium and potassium released from damaged muscle). The potassium from damaged cells then diffuses into the general supernatant and the potassium-level around healthy cells is lowered, explaining the lessened 'mortality' during the second day. Thereafter, the slow rise in 'mortality' may be due in part to the progressive deficit of essential nutrients as they are utilized by the tissues. The potassium contents of salt solutions and supernatants commonly used in tissue culture are as follows: Eagle's solution, 4·09 m.equiv./l.; Dulbecco's, 4·2 m.equiv./l.; Osgood's, 5·2 m.equiv./l.; Gey's, 5·25 m.equiv./l.; Earle's, 5·4 m.equiv./l.; Melnick's, 5·6 m.equiv./l.; Hanks's *T.C.* 199 and Scherer's, 5·84 m.equiv./l. The potassium-level in several of them is above the physiological limits in humans and in the range which, according to the observations in this communication, causes a poor initial functional survival of hearts in culture.

Fig. 2*a* shows a comparison of the effects of the same high (6·6 m.equiv./l.) potassium supernatant on the electrical activity of 6-day chick embryo whole hearts, isolated right atria and isolated right ventricles in culture. Atria have the best, whole hearts next best and ventricles the poorest initial 'survival'. Atria and whole heart 'survival' differs only in the first four days in culture. The poor 'survival' of ventricles is not due to nutritional difficulties because of 'large' muscle bulk, for whole hearts have a larger bulk, nor to the relative amount of tissue damaged during explantation, for atria have a higher relative damage. It may result from the sensitivity of impulse-generating and conducting tissue to high potassium-levels since whole heart and isolated atrium have a higher proportion of such tissue than isolated ventricles. However, the best explanation for the poor 'survival' of ventricles in high potassium supernatants is that, since the isolated right atrium and whole heart have previously established initiating foci for contraction and the isolated right ventricle has to establish a new focus, the new focus is more sensitive to high potassium-levels than the older established foci.

Fig. 2*b* is similar to Fig. 1*b* and compares the daily 'mortality' percentages for whole hearts, isolated right atria and isolated right ventricles maintained in the same high-potassium (6·6 m.equiv./l.) supernatant. The lowest 'mortality' occurs during the second day, possibly for the reasons postulated in the discussion of Fig. 1*b*. Thereafter, the 'mortality' rises at a faster rate than for hearts in Fig. 1*b*. The whole-heart series shows the best and the ventricles the poorest relative 'survival' behaviour. Further studies of the factors affecting the 'survival' of hearts in culture is being undertaken.

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Measurement of the Intrahepatic Shunted Flow of Blood in Cirrhosis of the Liver

THE most characteristic change in the hepatic circulation in cirrhosis of the liver is the appearance of pathological anastomoses in intrahepatic and extrahepatic blood vessels. The formation of intrahepatic and extrahepatic shunts markedly decreases the flow of blood through sinusoids, and increases the disturbance of liver cell function and liver cell damage, becoming one of the etiological factors of hepatic coma. Since there is apparently no published report of the quantitative measurement of the shunted flow of blood in liver cirrhosis, we have devised methods of measuring the intrahepatic and extrahepatic shunted flow in clinical cases. In this communication, the methods are presented for measuring the intrahepatic shunted flow of blood in cirrhosis of the liver. By these methods considerable amounts of intrahepatic shunted flow of blood is demonstrated in moderate and advanced liver cirrhosis.

The intrahepatic shunted flow of blood is measured either by the single administration method or by the continuous infusion method.

Single administration method. After the injection of an aqueous solution of 0·5 gm./kgm. body-weight of galactose in a single dose, blood is taken at successive intervals from the hepatic vein by means of hepatic venous catheterization and also from an arterial indwelling needle previously laid in the brachial artery. Quantitative analysis of galactose of these blood samples and urine is determined by a modified Fischer's[1] method. In normal subjects, at first, the peripheral arterial concentration curve shows the linear downward course, and hepatic venous concentration parallels the peripheral concentration, finally reaching zero level (first phase). When afterwards the amount of galactose entering the liver decreases below the maximal removing capacity of the liver, the peripheral concentration curve takes the shape of a simple exponential curve, and hepatic venous concentration remains at zero level (second phase). The hepatic flow of blood is calculated from the disappearance constant of the peripheral concentration in the second phase (k_2), the volume of distribution (V), and average urinary clearance of galactose (U_{cl}), according to the following modified equation of Waldstein[2]:

$$F = \frac{k_2 V - U_{cl}}{\left(1 - \frac{0{\cdot}4 \times \text{hæmatocrit}}{100}\right)} \times \frac{1}{\text{surface area}}$$

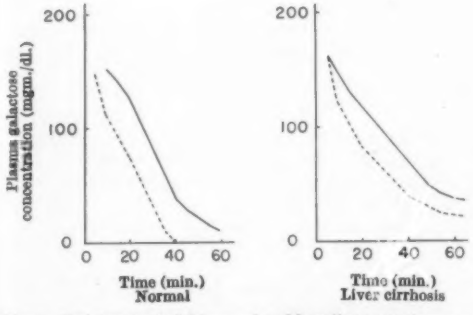

Fig. 1. Peripheral arterial (———) and hepatic venous (– – – –) galactose concentration curves by single administration method in a normal subject and in a patient with advanced liver cirrhosis

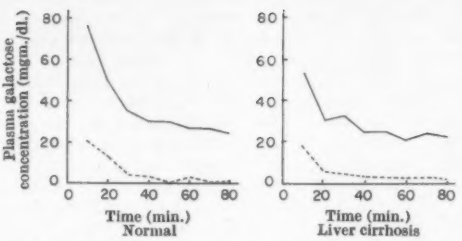

Fig. 2. Peripheral arterial (———) and hepatic venous (– – – –) galactose concentration curves by continuous infusion method in a normal subject and in a patient with mild liver cirrhosis

When there is an intrahepatic shunt between portal and hepatic veins in liver cirrhosis, the concentration in the hepatic vein does not drop to zero in the second phase, that is, galactose flows into the hepatic vein through the shunt, and the hepatic venous concentration curve in this stage takes the form of a simple exponential curve, too (Fig. 1). Therefore, in the presence of an intrahepatic shunt, the above equation does not give the total amount of blood flowing through the liver, but gives the amount of blood which enters the sinusoids in unit time, namely, effective hepatic flow of blood. On the same principle as in the Diodrast or *para*-aminohippuric acid clearance in renal physiology, total and shunted hepatic flow of blood can be calculated from effective hepatic flow of blood ($EHBF$) and the extraction ratio in the second phase (E).

$$\text{Total hepatic flow of blood } (THBF) = \frac{EHBF}{E}$$

$$\text{Shunted hepatic flow of blood } (SHBF) = THBF - EHBF$$

Continuous infusion method. After a priming dose of 10 gm. of galactose, constant infusion of galactose is made at a rate that does not exceed the maximum removing capacity of the liver, which is usually 200 mgm. per min. The curves become horizontal after 40 min. (Fig. 2). The total hepatic flow of blood is calculated on the basis of Fick's principle[3].

$$THBF = \frac{I_m - U_m}{(P - H)\left(1 - \frac{0{\cdot}4 \times \text{hæmatocrit}}{100}\right)} \times \frac{1}{\text{surface area}}$$

(I_m is galactose infused per min., U_m galactose excreted into urine per min., P peripheral plasma concentration, and H hepatic venous plasma concentration.) On the same principle as by the single administration method, the effective and shunted flow of blood is calculated:

$$EHBF = THBF \times E$$

$$SHBF = THBF - EHBF = THBF\ (1 - E)$$

In normal subjects the extraction ratio was in the vicinity of 100 per cent, and there was no estimated intrahepatic shunted flow of blood. In four cases with mild liver cirrhosis, the shunted flow of blood was 0–155 c.c./min./sq.m. or 0–20 per cent of the total hepatic flow of blood. In eleven cases with

moderate or advanced cirrhosis, on the other hand, the shunted flow of blood was calculated at 62–867 c.c./min./sq.m., or 18–78 per cent of the total hepatic flow. On the average, about half the total hepatic flow by-passed the liver in liver cirrhosis. Depressed extraction ratio was also noticed in two out of four cases with viral hepatitis in the icteric stage. Therefore, the intrahepatic shunted flow of blood calculated in this experiment is generally considered to include not only the flow of blood through the anatomic shunt, but also the functional shunted flow of blood due to hepatic dysfunction.

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Inhibition of Monoamine Oxidase by Benactyzine *in vitro* and *in vivo*

Vojtěchovský[1] has found recently that large doses of benactyzine (the diethylaminoethyl ester of benzylic acid) exert a psychotogenic effect. In doses of 40–200 mgm. it reduces the excretion of 5-hydroxyindole acetic acid in the urine to unmeasurable values[2]. These effects manifested themselves simultaneously. We suspected that benactyzine inhibits the monoamine oxidase which participates in the inactivation of serotonin[2]; and to confirm this hypothesis we tried to follow the effect of benactyzine on monoamine oxidase *in vitro* and *in vivo*.

For estimation of the activity of monoamine oxidase we used a 33 per cent homogenate of rat liver and 50 per cent homogenate of rat brain. The activity was determined using the modified method of Sjoerdsma *et al.*[3]. Instead of a decrease of serotonin we estimated production of 5-hydroxyindole acetic acid. After deproteinization of the homogenate with hydrochloric acid, we used the method of Udenfriend *et al.*[4], and the type of inhibition was determined by using Lineweaver and Burk's method[5]. Inhibition of monoamine oxidase *in vivo* was investigated in a homogeneous group of seven white rats, Wistar strain, weighing 200–250 gm. Benactyzine was administered subcutaneously in doses of 35 mgm. per 100 gm. body-weight. Normal values were obtained in a similar group of seven animals to

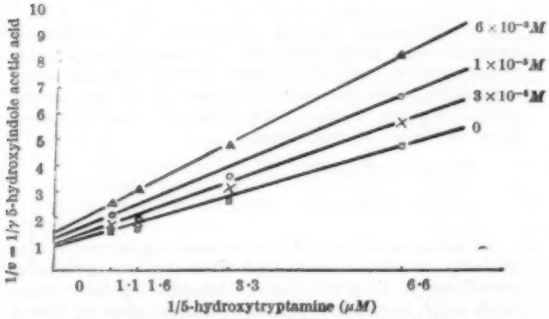

Fig. 1. Lineweaver–Burk plot of benactyzine inhibition as a function of 5-hydroxytryptamine concentration

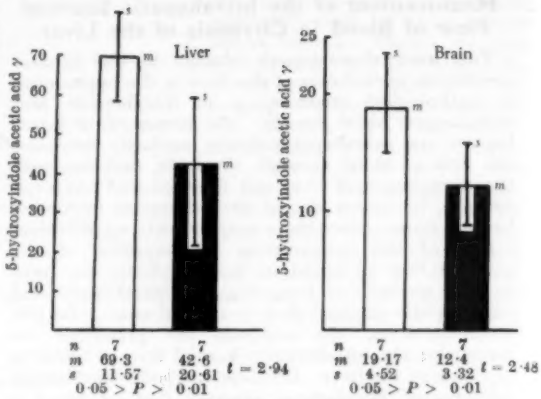

Fig. 2. Activity of monoamine oxidase *in vivo*

which no benactyzine was administered. The animals were followed up for 60 min. following the dose of benactyzine and were afterwards killed.

After homogenizing the brain and liver of both groups, monoamine oxidase activity was estimated[4].

Our experiments showed that benactyzine in concentrations of 4×10^{-3} *M* inhibits monoamine oxidase by 29 per cent, 4×10^{-4} *M* not at all; under similar conditions, 4×10^{-3} *M* 'Marsilid' inhibits it by 40 per cent and at concentrations of 4×10^{-4} *M* by 9 per cent. Benactyzine is thus a weaker inhibitor than 'Marsilid'. As shown by Fig. 1, benactyzine acts as a non-competitive inhibitor. Fig. 2 illustrates the inhibition of monoamine oxidase *in vivo*. It presents average values of the activity of the enzyme in the liver and in the brain; normal values, white columns; after benactyzine, black columns. Fig. 2 shows the variation of values and their statistical significance. Benactyzine when used in the above doses inhibits *in vivo* the monoamine oxidase in rat liver by 38·5 per cent on an average, and in rat brain on an average by 40 per cent. The results are somewhat distorted by the fact that two animals were resistant to benactyzine; thus they showed no signs of intoxication, and their behaviour did not change. In these animals no inhibitory effect was revealed in the organs investigated. This seems to us to explain also the great variations of values following the administration of benactyzine.

From our experiments it appears that benactyzine is a non-competitive inhibitor of monoamine oxidase *in vitro* as well as *in vivo*.

We are indebted to Mrs. J. Doubravová and Miss V. Kordová for technical assistance, and to Prof. V. R. Pickles, University of Sheffield, and to Sandoz, Ltd., Basle, for generous gifts of 5-hydroxytryptamine.

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Antigen-induced Damage to Isolated Sensitized Mast Cells

THERE is now quite convincing evidence that damage to mast cells always occurs when histamine is released during anaphylaxis. Thus damage to these cells is known to occur after active or passive sensitization either when antigen is injected in the intact animal or when a piece of sensitized tissue is incubated with the specific antigen[1]. However, all the evidence is derived from experiments made with tissues containing not only mast cells but also a mixed cell population, and therefore it is not possible to know whether damage to the mast cells is a direct consequence of the antigen–antibody reaction or whether antibodies are located on or in the mast cells.

We present here the results of some experiments performed in order to find out whether isolated mast cells would react to the specific antigen. Wistar rats of both sexes and weighing 150–250 gm. were used. The animals were sensitized by injecting 1 ml. of horse serum plus 1 ml. of *Haemophilus pertussis* vaccine containing 20,000 million organisms intraperitoneally and were used twenty-five days later. Mast cells were isolated by a method similar to that described by Padawer and Gordon[2]. The animals were bled under ether anæsthesia and 2 ml. of saline (sodium chloride 0·85 per cent) containing 50 μgm. heparin per ml. were injected into the abdominal cavity, and gentle massage of the gut through the abdominal wall was performed for about 1 min. The abdominal wall was then incised along the mid-ventral line and the fluid from the abdominal cavity collected with a pipette. This was carefully layered over 1·5 ml. of a 30 per cent water solution of bovine serum albumin (fraction *V*., Armour) and centrifuged in an International refrigerated centrifuge model *PR* 1 at 900 r.p.m. for 2 min. The albumin solution was then removed with a pipette, avoiding contamination with interface cells, diluted to 20 ml. with Tyrode solution and centrifuged at 1,000 r.p.m. for 5 min. This last operation was repeated once more and finally the deposit in the bottom of the tube was resuspended in 0·2–0·3 ml. Tyrode solution. This procedure resulted in a suspension containing 90–98 per cent of mast cells most of which were well preserved. These cells were then studied by aid of phase contrast microscopy either under dry magnification (× 800) or with oil immersion objective (× 1,000). A drop of the mast cell suspension was placed on a slide and covered with a square coverslip two sides of which were sealed with melted paraffin. A small well of paraffin was then made at each free side of the coverslip in the same way as was described by Strangeways and Canti[3], the preparation kept in a warm stage at 37° C., and a suitable group of well-preserved mast cells selected for observation. Horse serum heated at 37° C. was then placed in one free side of the coverslip and sucked on to the cells with a piece of filter paper. Usually the mast cells stood still when the antigen was sucked through, although sometimes many were washed away. As a control to these experiments the effect of horse serum on non-sensitized mast cells was also observed.

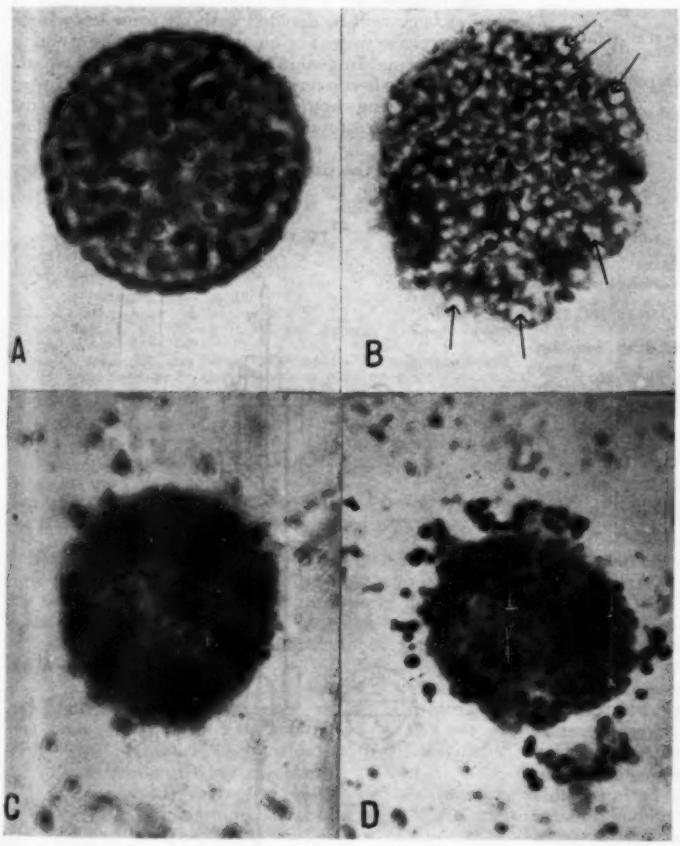

Fig. 1. *A*, Isolated sensitized mast cell before contact with antigen. Phase contrast (× *c.* 666); *B*, the same cell after contact with antigen. Arrows point to vacuoles (× *c.* 666). *C*, Isolated mast cell after fixation with 1 per cent formaldehyde and stained with toluidine blue (× *c.* 533). *D*, Isolated mast cell after contact with antigen. Note granules outside the cell. Fixed and stained as stated above (× *c.* 533)

In these conditions the effect of the antigen on sensitized mast cell was a very striking one. A few seconds after the antigen had been sucked on to the cells there was a slight increase in cell size followed by a sudden appearance and disappearance of successive vacuoles which made the cell look as if it were 'bubbling'. Sometimes it was possible to observe extrusion of the granules, which occurred, so far as could be observed, without damage to the cellular membrane. This phenomenon was very rapid, but was slowed down by low temperature and could be more easily followed at 25° C.—a temperature at which the phenomenon took as long as 1 min. to complete. When cell change was no longer seen, the cell had lost its regular contour, the granules had become more apparent and large empty spaces could be seen among them (Fig. 1). However,

we were not able to see any alteration in the form of the mast granules, most of which remained inside the cell. This phenomenon is very like that produced by compound 48–80 on rat mast cell[4]. The very conspicuous damage induced to mast cells in anaphylaxis[1] led us to propose the hypothesis that mast cells might contain antibodies[1]. Later on, Humphrey and Mota[5], concluded that the antigen–antibody reaction in anaphylaxis must occur in, or very near, the mast cells. However, at that time no direct proof could be produced that the mast cells contain antibodies. Our present results showing that washed isolated mast cells taken from sensitized animals are still able to respond to the specific antigen strongly suggest that mast cells contain antibodies and that damage induced to these cells in anaphylaxis is the consequence of an antigen–antibody reaction occurring in the mast cell itself. How the antigen–antibody complex once formed damages the cell is a challenging problem. However, there is some evidence that antibodies are located on the cell surface[5], and if it is assumed that γ-globulin is one of the protein constituents of the cell membrane, its combination with antigen could disturb the molecular equilibrium of the membrane and in so doing start the process of cell damage. Further experimental work is needed on this point.

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'Weightless' Micro-electrodes for recording Extracellular Unit Action Potentials from the Central Nervous System

In order to record the activity of single nerve cells in the mammalian central nervous system it is necessary to maintain the tip of the recording electrode (a micropipette or metal needle) within some 30μ of the cell body[1]. The skeleton of the animal is usually rigidly clamped to a heavy metal base and in many experiments this base also carries the micro-electrode upon a rigid support. Unfortunately the exposed central nervous system expands with every beat of the heart and also with exhalation of air from the lungs. Because such movements can make records of unit activity difficult or impossible to obtain, a technique has been developed[2,3] for recording from cells in the cerebral cortex in a rigidly enclosed cranial cavity. This system has proved so successful that lengthy records can be obtained from unanæsthetized, mobile animals. There are, however, limitations to this technique. It is not readily adaptable to exploration of a wide area of cortex; moreover, it cannot be applied to an examination of the spinal cord or brain-stem. For this reason we have developed the 'weightless' micro-electrode described below.

The principle of the device illustrated in Fig. 1 is to suspend the micro-electrode (with its tip appropriately placed) from a spring which is sufficiently light to allow the electrode to follow movements of the nervous system. A weak spring *S* is soldered at the upper end to a length of brass rod *R*; the lower end of the spring is soldered to a trace *T* which encircles a strip of thin celluloid *C* carried on a transparent 'Perspex' collar fixed on the end of the protective glass tube *G* that encases the whole assembly. The lower part of the diamond-shaped trace *T* is soldered to a chlorided silver wire; when the micropipette *M* is screwed into the conical-spring *CS* the chlorided silver wire dips into the saline that fills the butt of the electrode. The trace *T* also carries two central prongs *P* so arranged that they are in line and separated at the tips by a distance *d* where *d*–(celluloid thickness)≏1 mm. Electrical connexion with the cathode follower input is made through the whole metallic system supporting the weight of the micro-electrode.

The dimensions of the system are unimportant and cannot very well be decided without prior knowledge of the average weight of an electrode and the strength of the spring. In general, the electrodes used should be as light as is convenient for handling; the mass of trace and spring should be kept small, and the spring itself should be stretched to at least 150 per cent of its resting length by addition of the electrode. The dimensions of the instrument which we are using are provided in Fig. 1.

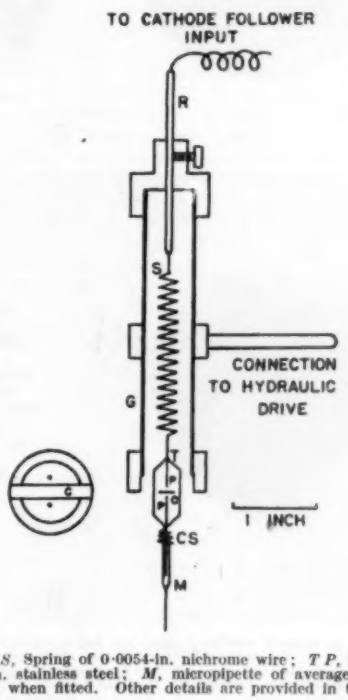

Fig. 1. *S*, Spring of 0·0054-in. nichrome wire; *T P*, trace of 0·0075-in. stainless steel; *M*, micropipette of average weight 70 mgm. when fitted. Other details are provided in the text

After attaching a particular micro-electrode, the electrode is weighed by raising or lowering R in relation to the upper 'Perspex' cap until the prongs P of the trace just ride clear of the celluloid gate C. The whole assembly may now be attached to an appropriate drive by a third 'Perspex' collar encircling the tube G and swung into position over the exposed nervous system. During insertion of the micro-electrode, as the whole carriage descends and the electrode touches the surface the celluloid gate comes in contact with the lower prong P. Insertion is continued until the micro-electrode's tip encounters a cell suitable for recording. If possible, it is desirable to descend 50–100μ below the optimal recording position; when the drive is reversed and an attempt is made to withdraw the electrode, the celluloid gate loses contact with the lower prong of the trace. Considerable movement of the whole carriage now leads to very small movements (in the same direction) of the micropipette, which is 'floating'. In this way the desired cell can be found again and, ideally, recording should be begun only when the celluloid gate is half-way between the two prongs. An instrument of this form must be designed to meet the owner's particular requirements. Our own carriages permit $\pm$200μ upward or downward movement of the nervous system and allow $\pm$2 mm. lateral movement of the preparation without loss of the record from a single unit. Lateral movements of 2 mm. can be made without causing any detectable alteration in amplitude of recorded spikes. This system completely eliminates the periodic variations of spike height which are due to respiration and the pulse in the fully exposed brain or brain-stem, and allows one to record from single units (deeper than about 100μ) for 6 hr. or more.

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HÆMATOLOGY

Bacterially Mediated False-Positive Reactions in Lytic Blood-Typing Tests

In blood-typing tests which employ lytic techniques, false-positive reactions can be brought about by the use of improperly rinsed and improperly dried test-tubes that still contain residues of acid or detergent or traces of water. In the course of performing routine blood-typing tests on citrated samples of cattle blood sent into this laboratory for blood-group analysis[1], we have encountered certain false-positive reactions which cannot be attributed to the use of dirty tubes. Rather, they appear to be of the order of true antigen–antibody reactions.

It is observed that the red cells in certain infected or bacterially contaminated samples of cattle blood are modified or transformed in such a manner that they react with certain natural antibodies which do not react with and cannot be absorbed by unmodified cattle red cells. These particular natural antibodies seem to be universally present, in varying concentrations, not only in virtually all our cattle blood-typing reagents[1] but also in fresh rabbit serum which is used as complement in the cattle tests.

We believe that these bacterially mediated transformations of cattle red cells may be closely akin or identical to the well-known transformations of human red cells—the Thomsen hæmagglutination phenomenon—brought about by certain bacteria or their filtrates[2]. At present, the main difference seems to be in the manifestation of the serological reactions. Transformed human red cells are said to be panagglutinable. That is, they agglutinate in virtually any serum including the autologous serum. Transformed cattle red cells are rarely if ever panagglutinable, an observation made long before us by Friedenreich[2]. This is in keeping with the observation that unmodified cattle red cells rarely exhibit agglutination even when sensitized with multiple doses of blood-typing antibodies—a fact which has necessitated the use of lytic techniques in the cattle studies[3].

Transformed cattle red cells, when sensitized with sufficient natural antibodies, are usually lysed in the presence of an appropriate complement (C') such as that in fresh rabbit serum. Whether they will be lysed by the action of natural antibodies and C' seems to depend largely on the degree of transformation and upon the concentration of natural antibodies not only in the various dilutions at which our blood-typing reagents are used but also on the concentration of natural antibodies in fresh rabbit serum.

When the red cells are what we shall refer to as maximally transformed, they are essentially panhæmolytic. That is, they become lysed in the tests with each reagent and also in the complement controls. Therefore, the complement controls alone are usually adequate in screening maximally transformed cattle red cells. Such controls are not, however, adequate in screening minimally transformed cattle red cells, and herein lies the fallacy in the notion that complement controls in conjunction with saline controls are sufficient to detect those samples of blood which are unfit for typing in lytic tests. The complement controls alone will not do this because, in our experience, the concentration of natural antibodies in pooled rabbit serum is usually not above a threshold necessary to bring about lysis of minimally transformed cattle red cells during the ordinary time-course of the blood-typing tests. When minimally transformed red cells are lysed in the complement controls, the lysis may not begin to make its appearance until the final reading (check-reading) of the tests and it is usually of a very weak degree. Furthermore, in the absence of reactions brought about by the specific blood-typing antibodies, the minimally transformed red cells seem to maintain their stability as well as or even better in tests with the majority of our reagents than they do in the complement controls. We suspect, as indicated by titrations, that certain constituents in the serum of the reagents exert a protective influence on the transformed red cells.

Some of our reagents, however, are rather richly endowed with the offending natural antibodies, and it is in the tests with such reagents that lysis of minimally transformed red cells takes place in spite of the protective influence of the serum. Such reactions can simulate closely those brought about by the specific blood-typing antibodies, thereby

providing a previously unsuspected and a particularly subtle kind of false positives in lytic tests.

The observations which led us to the discovery of false positives of this order is another story which we hope to publish elsewhere along with results of transformation of cattle red cells brought about not only by various bacteria isolated from cattle blood samples but also with borrowed cultures of organisms known to transform human red cells. In the meantime, it may be well to mention at least one of the steps which we have taken in order to detect those samples in which the red cells may be minimally transformed. In addition to being cognizant of those reagents which are richly endowed with natural antibodies and are thereby capable of producing false-positive reactions with minimally transformed red cells, we employ, as a control, a bovine serum which contains no specific blood-typing antibodies but does contain natural antibodies in sufficient concentration to react with minimally transformed red cells. Red cells which become lysed in this reagent are regarded as transformed and are thereby subjected to close scrutiny with respect to their reactions with the regular blood-typing reagents. When there is any doubt regarding the latter reactions, antibody-absorptions can be performed to distinguish those reactions which may be brought about by natural antibodies rather than by the specific blood-typing antibodies.

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Antithromboplastin in Human Serum and Plasma

The existence of antithromboplastin as a blood coagulation factor has been well documented (for example, see ref. 1). While it is likely that antithromboplastin plays a part in the prevention of blood coagulation in the body in health, it does not appear to have been characterized physiologically. Berry[2] has indicated a suitable technique for its demonstration which we have applied with slight modifications in detail only to whole serum, whole plasma (platelet-free) and also to the electrophoretic fractions of serum and plasma. These fractions were eluted by mechanical squeezing from strips cut from pieces 36-cm. wide of Whatman No. 3 paper used in a 'fakir's mattress' type of electrophoresis apparatus[3]. Our technique was, basically, to incubate serum, plasma or the electrophoretic fractions with human brain thromboplastin and calcium chloride at 37° C., and at known intervals to add an aliquot of this incubation mixture to a substrate platelet-free plasma and note the time it takes to clot. A saline control repeatedly gave coagulation times of about 20 sec. (shown as 100 units on the ordinate in Fig. 1). It should be noted that this technique demonstrates antithromboplastin active only against the extrinsic tissue type of thromboplastin and not against the thromboplastin formed by blood undergoing the natural coagulation mechanisms (intrinsic thromboplastin).

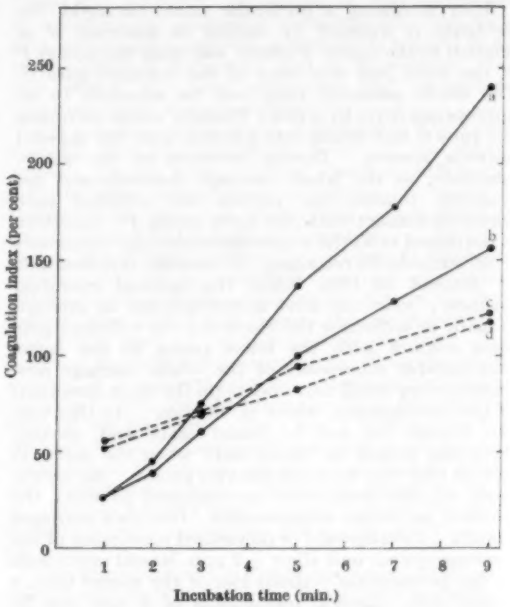

Fig. 1. Curves (*a*) and (*c*) show the progressive retardation effect of plasma and serum respectively on brain thromboplastin. Curves (*b*) and (*d*) show the effect after the addition of thrombin. The units of the ordinate are percentages. All coagulation times, including those in Table 1, were expressed as percentages of the saline control, so as to overcome differences due to slight day-to-day variations in the latter

Serum, when thus treated, showed an initial acceleration followed by a progressive lengthening of coagulation times (curve *c*). It is this progressive inhibition of the thromboplastin, claimed by Berry[2] to be due to an antithromboplastin, which can be estimated semi-quantitatively by the gradient of the curve; steeper curves indicating greater amounts of activity.

Plasma showed a greater level of activity than serum (curve *a*). The initial acceleration of coagulation in both cases is probably due to the presence of factor VII, since it is absent after absorption by aluminium hydroxide[3]. The eluates from the α_1-globulin region of serum show an accelerating effect (that is, a coagulation index less than the 100 per cent control-level); see Table 1, column *a*. This, again, is probably due to factor VII, which has been shown to migrate in this region[4]. We cannot explain the accelerating effect seen in the β-globulin region. It is possible that it is associated with lipoproteins, many of which migrate in this zone.

Retarding effects appear in the α_2-globulin and the γ-globulin regions in both serum and plasma (columns *a*, *c* and *d*). Only in plasma is the retarding effect from both these regions progressive (column *d*) and therefore probably due to antithromboplastins. From columns *c* and *d* it can be seen that there is an increase in coagulation times when the fractions are incubated for 2 min., and then for 1 hr. In serum, this progressive retardation is seen only in the α-globulin region (column *c*). This suggests that there are two circulating antithromboplastins; one, which migrates in the α-globulin region, present in serum

Table 1. Coagulation Times (Percentage of Normal) of Electrophoretic Fractions

Electrophoretic fractions		Serum	Serum plus thrombin	Difference in times after 2- and 60-min. incubation	
Stainable protein	Fraction No.	(*a*)	(*b*)	Serum (*c*)	Plasma (*d*)
Albumen	1	87	35	−7	3
,,	2	81	26		
,,	3	76	34	19	4
α_1-Globulin	4	84	31		
None	5	84	62	14	36
α_2-Globulin	6	98	124		
,,	7	109	117	13	22
β-Globulin	8	80	30		
,,	9	72	32	4	19
None	10	81	26		
γ-Globulin	11	107	26	−6	37
,,	12	132	23		
,,	13	125	35	−6	47
,,	14	100	34		

The difference in times in columns (*c*) and (*d*) is given in the sense: (time after 60 min. incubation) minus (time after 2 min. incubation). The fractions in columns (*c*) and (*d*) were paired in order to conserve materials.

and plasma, and another, migrating in the γ-globulin region, in plasma only.

Further evidence for two antithromboplastins was shown by the effect of adding a preparation of thrombin to the incubation mixtures. The amount of thrombin added was that which shortened the coagulation time of a saline – thromboplastin – calcium chloride mixture from 28 to 16 sec. When added to serum during incubation, no effect on the steepness of the curve was produced (curve *d*), but there was a marked decrease in antithromboplastic activity when thrombin was added to plasma (curve *b*).

When thrombin was added to the electrophoretic fractions during incubation, the retarding action in the γ-globulin region was neutralized (column *b*). The presence of long coagulation times in the α_2-globulin region in this column cannot, however, be assumed to be due entirely to antithromboplastin. It is probably also partly due to antithrombin, which is known to migrate in this region[4]. (Note that the activity in this region in column *b* exceeds the 100 per cent control level—a somewhat unexpected finding.)

The above findings suggest to us that there are two circulating antithromboplastins in plasma. One of them (the γ-globulin one) is neutralized by thrombin and presumably also during normal coagulation processes; and the other one in the α-globulin region remains unchanged throughout and is present in serum.

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Determination of Calcium in Blood Serum by Atomic Absorption Spectroscopy

Although many methods have been described for the determination of calcium in blood serum, they give results which vary over a range of nearly 20 per cent[1,2], and there is still no agreement as to which method is the most reliable. In general, the values given by the flame photometric methods are 3–4 per cent higher than those obtained from the classical oxalate–permanganate titration, which in turn are about 10 per cent higher than those given by complexometric and colorimetric methods, while the values given by spectrographic emission methods are lower still.

A completely new approach to the problem is afforded by the development of the technique of analysis by atomic absorption spectroscopy[3,4], and, following the development of a simple method[5] for determining serum magnesium by this means, I have now investigated the determination of serum calcium also.

With the same equipment as in the earlier work, but using a calcium hollow-cathode tube and passing the radiation through the lowest part of the air-acetylene flame, just above the zone of unburnt gas, accurate measurements of the absorption of the calcium resonance line (4227 Å.) were possible in solutions containing 5–15 p.p.m. calcium. The presence of phosphorus lowers the absorption markedly; but this can be entirely overcome by the addition of large concentrations of strontium chloride or of the disodium salt of ethylenediamine tetraacetic acid. Provided a rich acetylene–air mixture is used, the protein in serum nullifies the effect of the phosphate present, and for routine purposes measurements can be made on serum diluted 10- or 20-fold with water. Since only 2·5 ml. of solution are required for duplicate calcium determinations, less than 0·2 ml. of serum is required for the estimation of this metal. Magnesium, even in quantities far in excess of its concentration in serum, is without effect on the calcium absorption. The sodium and potassium present in serum have only a small influence on it, which is allowed for in any event by standardizing the instrument with solutions of calcium containing about the same concentration of sodium as do the serum solutions.

Table 1 shows calcium contents of sera obtained in the following ways: (*a*) The calcium was precipitated as oxalate, and the supernatant liquid removed by draining after centrifuging. The precipitate was dissolved in a drop of hydrochloric acid and made

Table 1. Calcium Content (mgm./100 ml.) of Blood Serum

Serum No.	Type	(*a*) Calcium pptd. as oxalate	(*b*) Serum deproteinized	(*c*) Serum diluted with water	Oxalate-permanganate titration
1	Bovine*	11·15	11·35	11·0	11·1
2*A*	Equine†	8·5	8·95	8·6	9·1
4	Human	—	10·4	10·2	9·9
5	,,	9·8	9·85	9·35	—
17	,,	—	10·35	9·75	—
18	,,	—	8·85	8·6	—
20	,,	—	9·7	9·9	—
22	,,	—	10·3	10·4	—
9	,,	6·9	6·95	—	—
12	,,	8·1	8·2	—	—
15	,,	7·05	6·85	—	—
19	,,	—	9·35	—	8·8

* Freeze-dried serum.
† This sample had stood in the refrigerator for several months and was centrifuged to remove the precipitate which had formed.

up to 5 ml. with strontium chloride solution (2,500 p.p.m. strontium). (*b*) The serum solution was deproteinized by the addition of trichloracetic acid containing strontium chloride and measurements were made on the supernatant liquid after centrifuging. (*c*) The serum was diluted directly with water to 5 ml.

It will be seen that there is good agreement between the values obtained for the calcium content of serum by these three variations of the atomic absorption technique, and that in general the results are slightly higher than the values obtained by the oxalate–permanganate titration. There is a small but significant difference between the results obtained by methods (*a*) and (*b*), which is of the magnitude to be expected from a combination of two factors, namely: (1) the failure of the oxalate buffer to precipitate the last 0·8–2·0 per cent of serum calcium[6] and (2) the decrease in volume, estimated at 0·5 per cent, of the serum solution after deproteinization in method (*b*).

It is clear that method (*b*) is an accurate procedure for determination of calcium in serum, while method (*c*) gives very rapidly results which may be of sufficient accuracy for many purposes.

A fuller account of this and the earlier[5] work will be submitted to *Spectrochimica Acta*.

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PATHOLOGY

Cirrhosis of the Liver induced in Rabbits by Gastric Instillation of 3-Monohydroxycholanic Acid

Daily administration to rabbits of a desiccated whole bile preparation rapidly gives rise to cirrhosis of the liver[1]. Later findings suggested that the effect was linked to the activity of bile acids[2] although several of the first tested and more common variants of them showed only a feeble liver damaging effect when used according to the present method[2].

On the basis of further analysis of the dried bile preparation, several variously substituted cholanic acid derivatives have been investigated. Of the bile acids so far tested 3-monohydroxycholanic acid (lithocholic acid) has been found highly effective. Daily gastric instillation to rabbits (mean weight 2·5 kgm.) of 10 ml. of a 0·5 per cent water suspension of the sodium salt of this acid (L. Light and Co., Ltd.) induced cirrhosis of the liver in 10 of 11 animals within three months. In several control groups of equal size no cases of cirrhosis were found. Higher concentrations led to more extensive liver damage and death of the animals.

Massive fat infiltration was not observed. The extent of necrosis varied with the dose administered and the individual resistance of the animal. Hydropic degeneration of the liver cells was prominent. The ultimate picture was usually that of a finely nodular diffuse cirrhosis. A detailed report will be published elsewhere.

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Estimation of Mouse Tumour Blood Volumes employing a Radioactive Isotope Technique

Changes in radiosensitivity and growth-rate in a serially irradiated line of the mouse sarcoma 37 have been reported elsewhere[1]. The effect of oxygen concentration on radiosensitivity has been reviewed recently by Gray[2] and might have contributed to the results obtained with sarcoma 37. The reduction in growth-rate in the serially irradiated line may have been due wholly, or in part, to a less-adequate development of blood vessels and capillaries within the tumour—resulting in longer diffusion paths. The evaluation of the internal vascularity of control and irradiated-line tumours was undertaken in an attempt to elucidate the causes of the observed changes. Macroscopic observations of the tumours *in situ* (developing on the abdominal skin) indicated a comparable increase in size of the cutaneous vessels in control and irradiated-line tumours.

Evaluation of the internal vascularity was undertaken by counting the ionization events within excised tumours after blood-labelling with a suitable tracer. This technique was possible owing to the lack of necrotic regions and blood sinuses in these tumours below a weight of approximately 700 mgm. The radioactive isotope employed had to be bound to a relatively inert molecule incapable of diffusion through the capillary walls. Radioiodine (^{131}I) bound to human serum albumin and suspended in a buffered saline[3] conformed to these requirements. This preparation was kindly made available by the National Institute for Medical Research, Mill Hill, London, N.W.7. The activity was of the order of 350–400 μc. per ml. on preparation.

The solution was injected into a lateral tail vein of lightly anæsthetized mice. Owing to the small quantity available from each preparation (about 3·5 ml.), approximately equal numbers of mice from each group were injected from each batch. Some batches produced a marked anaphylaxis, consequently the volume injected varied between 0·25–0·15 ml. per mouse. 3–5 min. after injection were allowed for mixing before killing by cervical dislocation. The tumours were then immediately excised, blotted with No. 5 filter paper and weighed. A blood sample was taken from the heart, placed in a 'Perspex' chamber of about the same volume as the tumour and weighed. Activity counts were made by a scintillation counter on the tumour, blood sample and background. The weight of blood in each tumour was calculated from the resulting data.

The results of these measurements for 19 irradiated *B* line (*B*12) and 22 control tumours are shown in Fig. 1. Blood weights are plotted against tumour weights on a double logarithmic scale. The trend

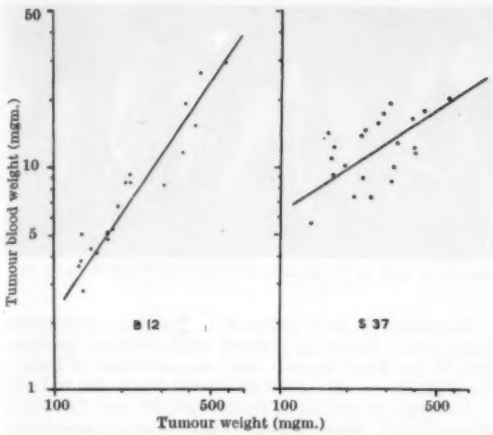

Fig. 1. Estimations of tumour blood proportions in sarcoma 37 and its serially irradiated line *B*12

lines for *B*12 and control data corresponded to slopes of 3/2 and 2/3 respectively. The scatter for *B*12 tumours was small, the correlation coefficient being 0·948—indicating a high degree of correlation (perfect functional relationship being unity). For control tumours a wider scatter was apparent, the correlation coefficient being 0·584—indicating the presence of a trend, but with a low degree of correlation.

The smaller blood volume values in *B*12 as compared with control tumours below a weight of about 350 mgm. (comparable to a tumour area of about 35 sq. mm.) suggest that the increase in time of tumour establishment experienced with this line may be due to a reduced vascular response by the host to the initial implant. A comparison of the trend lines and values for tumour weights greater than 350 mgm. indicates that vascularity is not a factor in explaining the reduction in growth-rate of established irradiated-line tumours.

The high degree of correlation between blood and tumour weights experienced with the *B*12 tumour results would appear to indicate that accurate evaluations of tumour blood content can be made by this technique.

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Synovial Proliferation induced by Polysaccharides

One of the characteristic histopathological changes that occur in rheumatoid arthritis is the formation of pannus. This synovial granulation tissue grows over the surface of the articular cartilage, and in so doing erodes the underlying matrix. If remission of the disease process occurs at this stage, then the resulting abnormal joint surfaces will eventually lead to the development of secondary osteoarthritic changes, irrespective of further exacerbations in rheumatoid disease. The development of synovial pannus is therefore of critical importance in the natural history of the disease process in a given joint, and the study of substances that are capable of producing a similar condition in experimental animals is of interest.

Jones and Carter[1,2] have shown that a proliferative synovitis can be induced in guinea pigs by the subcutaneous or intravenous injection of bacterial polysaccharide complexes. These findings raised the possibility of inducing a similar effect by the intra-articular injection of other polysaccharides the chemical nature of which was better understood, and consequently we have investigated the effect of intra-articular carrageenin, a polygalactose sulphate employed by other workers to produce connective tissue granulomas[2–5].

The polysaccharide solution in normal saline was sterilized by autoclaving, and then injected, under pentothal anæsthesia, through the antero-lateral approach into the femorotibial joints of young adult male rabbits. Following varying periods of time, the animals were killed by air injection and the synovial membranes dissected, fixed and stained. Cultures from all joints were made in Robertson's cooked meat or Brewer's thioglycollate media, so that all infected material could be discarded. The degree of synovial response to the polysaccharide was measured by the changes in either (*a*) the average number of cells per unit area or (*b*) the mitotic index, expressed as the number of mitoses/1,000 cells/per hr., following colchicine arrest.

After a single injection of 4 mgm. of carrageenin there is a wave of proliferation, the number of synovial cells per unit area being at a maximum on the tenth day, and then falling gradually. The effects of 0·2, 2·0 and 20 mgm. of polysaccharide per knee differed significantly in the degree of synovial proliferation. The effect of the highest concentration, however, was to elicit a much more marked influx of leucocytes and macrophages. In consequence, doses of between 2 and 3 mgm. of polysaccharide were used in further experiments, as with this dose-level proliferation was easily stimulated and wandering cells were at a minimum. Table 1 shows the variation of mitotic indices with time following a single intra-articular injection of 3 mgm. of carrageenin. The contralateral knee was used as a control, as no increase in mitotic index above normal limits was ever observed in these knees following injection of their treated pairs.

The results are expressed as the ratio of the treated mitotic index to the mean control mitotic index, which was 0·19 (standard error of mean 0·02).

Table 1

Days after injection	1	2	3	4	5
Treated / Control mitotic index	4·3	30·9	29·3	21·9	17·0

Parallel investigations of the levels of ribonucleic acid, deoxyribonucleic acid, phosphocreatine, diphosphopyridine nucleotide and the *in vitro* uptake of oxygen, lactate production and glucose utilization showed that their maximum changes occurred at about the same period. These findings will be reported in full elsewhere.

When carrageenin was injected weekly for several weeks, the resulting massive synovial proliferation was accompanied by areas of early pannus formation caused by the erosion of the matrix of the articular cartilage by the synovial granulation tissue. In

some rabbits, areas of small round cell accumulations were found in the proliferating villi, which resulted in a general histological picture very similar to that found in rheumatoid arthritis.

Other polysaccharides, with widely varying structures, were also compared for their effect upon synovial proliferation by the use of a balanced incomplete block design. Glycogen, fucoidin, chondroitin sulphate, dextrin, starch (soluble) and pectic acid were inactive but alginic acid and agar (Japanese) were significantly active, although less so than carrageenin. Prof. Choji Araki, Kyoto Technical University, Japan, very kindly supplied us with specimens of agarose and agaropectin, the two components of this agar. The former compound has little effect; but agaropectin is as active as carrageenin in the induction of synovial proliferation.

It is of interest that the two most active compounds both contain galactose. Carrageenin is a mixture of two polysaccharides, one containing sulphated galactose, the other sulphated galactose and 3 : 6-anhydro galactose chains. However, both constituents of agar contain galactose and 3 : 6-anhydro galactose[6], the active component, agaropectin, also containing small amounts of sulphate, glucuronic acid and pyruvic acid. It is unfortunate that little is known of the detailed structure of agaropectin.

Further work is in progress on the possible relationship between polysaccharide structure and the induction of synovial proliferation; but it is suggested that the experimental system reported here may have some use for the screening of therapeutic substances, such as steroids and their synthetic analogues, for anti-proliferative activity in synovial membrane.

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Anthelmintic Efficiency of Tetrachlorodifluoroethane against *Fasciola hepatica* in Sheep

Carbon tetrachloride and hexachloroethane are the most frequently used anthelmintics against *Fasciola hepatica*, the common liver fluke of sheep. These drugs are not always fully effective, and both at times produce severe toxic symptoms or death. Consequently, there remains the need for a safer and more effective agent active against liver fluke.

Fig. 1. Ascending necrosis of body of *F. hepatica* following treatment of sheep with 'Freon-112'

Inorganic[1,2] and organic[3,4] fluorine compounds have been shown to possess anthelmintic qualities, and it has been stated[5] that substitution of fluorine for chlorine in the methane series decreases toxicity.

Laboratory and field trials with 29 and 126 sheep, respectively, have shown that tetrachlorodifluoroethane ('Freon-112', E.I. du Pont de Nemours and Co. (Inc.), Delaware) is highly effective against mature *F. hepatica* at a dose-rate of 0·15 gm./lb. body-weight. Admixture with liquid paraffin to give a 50 per cent w/v mixture lowered the high freezing point and vapour pressure and gave a convenient solution for drenching. There was regularly a drop of 99–100 per cent in faecal egg count. No living flukes were found in 29 previously infested sheep when the livers were examined at an abattoir 19 days after treatment. The drug was administered by intra-abomasal injection to two sheep and intrarumenally to four with similar results.

The effect on immature flukes was variable, and ranged from a complete kill to very little reduction in numbers. The low toxicity of 'Freon-112' will permit higher dose-rates to be employed in future trials of its efficiency in acute fasciolosis.

It has been reported[6] that doses of 2 gm./kgm. per day for up to 33 days did not produce liver damage in rats. No pathological changes were found in the liver, kidney or myocardium following single doses of 0·75 and 1·0 gm./lb. given orally to sheep 24 hr. before slaughter. No clinical symptoms of toxicity were observed in more than 150 sheep in 5 different locations treated with 'Freon-112'.

Three sheep were killed 24, 48 and 72 hr. respectively after treatment, and the flukes and eggs were collected. A very strong contraction of the uterus was observed, and it appeared that all eggs had been extruded from the flukes. In some cases the whole body except the oral and ventral suckers and the extreme oral portion of the fluke was necrotic (Fig. 1). In others most of the body had disintegrated and vanished, but the suckers and oral part were still active. This ascending necrosis of the fluke body has been observed by us at only one of many examinations following treatment of infested sheep with carbon tetrachloride. 'Freon-112' produced an adverse effect on the development and hatching of fluke eggs collected from the gall bladder and then incubated

Table 1. Effect of 'Freon-112' on Fluke Eggs
Results given as percentage of total eggs examined after 2 weeks incubation

Sheep	Hr. after treatment	No development	Development started	Hatched
C 71	24	22	64	14
C 74	48	42	48	10
C 277	72	70	28	2

at 28° C. for 2 weeks. The intensity of this effect was increased with the length of the period during which the eggs had remained in the treated sheep (see Table 1). This is a useful residual effect.

Details of these experiments will be published in the *Australian Veterinary Journal*.

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Subnormal Level of Carbonic Anhydrase in Blood of Carcinoma Patients?

It was shown in a recent paper[1] that the zinc content of whole blood in cases of very different cancers was subnormal (a mean decrease of about 20 per cent being found). Various metalloproteins are known which contain zinc as an essential part[2]. It seems wise to devote careful attention to carbonic anhydrase, a substance present in relatively large amounts in red blood corpuscles and described in detail by Keilin and Mann[3].

If, when using the preparation method of these authors quantitatively, it should turn out that the quantity of zinc present in whole blood equals the quantity of zinc present in carbonic anhydrase prepared from this blood, one might characterize carcinomas by a subnormal level of carbonic anhydrase. Table 1 shows results of determinations of zinc carried out by analysing various blood fractions which Dr. H. J. van den Berg was kind enough to prepare. After centrifuging off the serum, hæmolysing the erythrocytes and precipitating hæmoglobin with ethyl alcohol and chloroform, the remaining carbonic anhydrase was precipitated with lead acetate. The determination of zinc was carried out spectrochemically[4].

According to Keilin and Mann, 50–60 per cent of carbonic anhydrase is occluded during the precipitation of hæmoglobin, which can also be seen from Table 1.

Table 1

	Weight (gm.)	Zinc content (p.p.m.)	Weight of zinc (gm.)	Weight of zinc (gm.)
Whole blood	201	5·7		0·00115
Plasma	102	0·9[5]	0·000097	
Leucocytes + water	1·25	25	0·000031	
Erythrocytes	105	9·9	0·00104	
		Total	0·00117	0·00115
Erythrocytes	105	9·9		0·00104
Hæmoglobin:				
First fraction	25	13·6	0·00034	
Second fraction	3	43·8	0·00013	
Lead precipitate	4	136	0·00054	
		Total	0·00101	0·00104

The lead precipitate cannot be decomposed without destroying the protein. For this reason the clear solution of carbonic anhydrase was dialysed against water, whereby according to Vikbladh[5] once again zinc ("loosely-bound zinc") is lost up to about 70 per cent. The dialysed solution, free from chloroform, alcohol and salts, was dried *in vacuo* at a low temperature (about 5° C.).

Table 2 shows the zinc balance.

Table 2

		Mgm. zinc
100 ml. of whole blood contains		0·65
The serum fraction contains		0·1
	remainder	0·55
60 per cent loss owing to co-precipitation with hæmoglobin	remainder	0·22
70 per cent loss owing to loosely bound zinc being dialysed	remainder	0·07
45 mgm. of impure carbonic anhydrase (containing 0·15 per cent zinc) prepared from 100 ml. of whole blood contain also		0·07

It appeared to be possible to increase the purity of the product: its zinc content varied between 0·25 and 0·35 per cent (Keilin and Mann, 0·33 per cent; other investigators, between 0·2 and 0·32 per cent).

The properties of the preparations are as follows:

(1) One of them appeared to contain 14·1 per cent nitrogen (Dr. F. J. Spruit); Keilin and Mann mention a value of 14·95 per cent.

(2) Paper electrophoresis (Dr. J. Voogd) showed the existence of two protein fractions (see also Bate-Smith[6]). The main fraction appeared to change proportionally with time into the second fraction, which has a smaller rate of migration and probably a larger molecular weight. This change appeared to be closely related to the zinc content of the carbonic anhydrase. It is known that zinc facilitates the formation of larger molecules. On the other hand, this effect of change was shown to be absent when all manipulations were carried out in the absence of oxygen. It is therefore probable that carbonic anhydrase is sensitive to oxygen.

(3) Semi-quantitative spectrochemical analyses showed the following results: 0·2–0·8 per cent potassium and also sodium; 0·7 per cent calcium; 0·1–0·2 per cent phosphorus; 0·05–0·1 per cent magnesium; 0·01–0·03 per cent copper; 0·01 per cent iron and 0·001 per cent manganese (a preparation made from ox blood appeared to contain twice as much of these elements, but only 8 per cent nitrogen).

The following hypothesis seems worth serious consideration. A subnormal level of carbonic anhydrase in carcinoma cases can have a serious effect on the metabolism. The transport of carbon dioxide can be hindered, and this is certainly the case in a tumour resulting in a process of fermentation[7]. Therefore in my opinion it would be worthwhile to study intensively the anti-carcinogenic activity of carbonic anhydrase.

Thanks are due to Dr. H. J. van den Berg, Dr. F. J. Spruit and Dr. J. Voogd for their valuable help as well as to Miss H. Savenije for her careful determinations of zinc.

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RADIOBIOLOGY

Inability of Thiotaurine to protect Mice against Ionizing Radiation

One of the theories proposed for explaining the protective role of certain substances on animals submitted to a lethal dose of ionizing radiation involves the capture of free radicals by the protective compound (scavenger action). As a consequence of this process the compound is chemically modified in the biological structures where it happens to be, which then survive the irradiation. If such a theory is correct a certain degree of correlation would be expected between the protecting activity of a substance and its lability towards radiation, unless a catalytic mechanism is involved.

Cystamine, one of the best-known biological protectors against radiation[1], is relatively resistant to radiation in solution. A low percentage of degradation products has been detected only after heavy irradiation[2]. Thiotaurine, a metabolic product of cystine and cystamine, has been recently found more sensitive than cystamine to an equal amount of X- and γ-rays[3].

The validity of the presumed correlation between radiochemical degradation and biological protection has been investigated by comparing the protective effects of thiotaurine and cystamine against a lethal dose of X-rays on mice. Three groups of 15 white male mice of the Swiss strain weighing 20 gm. were placed in a wooden box with a 'Perspex' cover in mixed groups of 15 animals and exposed to a dose of 600 r. in the space of 15 min. One group was the control: another was injected intraperitoneally with half the *LD*50 of cysteamine neutralized with *N* hydrochloric acid (cysteamine and cystamine have equal protective power[1]), 15 min. before irradiation. The third group received half the *LD*50 of thiotaurine[4] in the place of cysteamine. Under our conditions the *LD*50 for cysteamine and thiotaurine were respectively 0·27 gm./kgm. and 3·52 gm./kgm., that is, thiotaurine is tolerated much more than cysteamine. On the molar base, the thiotaurine injected represents an amount seven times higher than that of cysteamine.

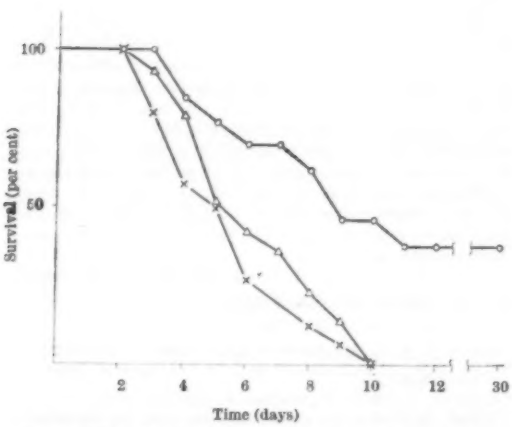

Fig. 1. Effect of cysteamine and thiotaurine on the survival of mice after exposure to X-rays. ×, 600 r.; ○, 600 r. and half the *LD*50 of cysteamine (0·135 gm./kgm.); △, 600 r. and half the *LD*50 of thiotaurine (1·760 gm./kgm.)

The results are shown in Fig. 1. It may be seen that all the thiotaurine-treated mice died within 10 days of irradiation, while little less than 50 per cent of the cysteamine-injected mice are still alive. This clear-cut result excludes any possible role of thiotaurine as a protective agent despite its relative lability towards radiations, and despite the large dose injected. This finding suggests that the correlation of the radiochemical degradation of a substance with its biological protective power is untenable. Moreover, it supports the view of those who believe that the 'scavenging action' plays only a minor part in the mechanism of the chemical protection[5].

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Divergence between Lethal Doses and Sterilizing Doses of X-Rays with Progressive Development in *Habrobracon* Females

In the present work I have determined the weakest link in an insect life-cycle and related this to the quantitative differences between the sterility and lethality doses when virgin females were irradiated at various developmental stages. The wasp used is known in genetic literature as *Habrobracon juglandis* (Ashmead). The females were chosen since it was believed that a non-sterile female would normally live and copulate with a son and afterwards produce both males and females. Critical stages for exposure were selected at significant changes in somatic and/or gonadic differentiation.

The stock, derived from a mother–son mating in 1952 of wild type Whiting stock No. 33, has maintained excellent egg production and viability for more than 270 generations. The small braconid wasp parasitizes various lepidopteran larvæ. *Ephestia kuehniella* (Zeller) caterpillars are the standard host employed. *Habrobracon* females usually mate once. Fertilized eggs develop into diploid females; unfertilized eggs develop parthenogenetically into haploid males. All rearing was done at 30° C., and samples of 20 wasps per stage were used.

Eggs or immature forms of known age were irradiated at the Marine Biological Laboratory, Woods Hole, Massachusetts (25 m.amp., 250 kV. peak, filtration ≡ 0·2 mm. copper). A constant target–tube distance of 23 cm. was maintained to produce 600 r./min.

Females were considered sterile if oviposition failed; or in the case of forms more than 6 days old, if oviposition ceased after the deposit of eggs preformed at the time of irradiation. The lethal dose is considered to be that X-ray exposure which prevented eclosion; consequently, death could occur between

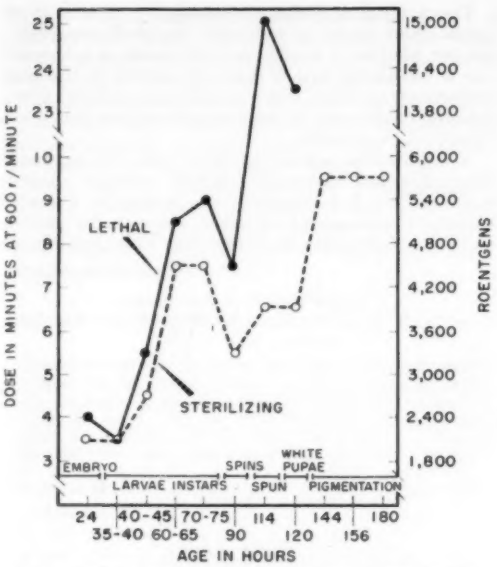

Fig. 1. Sterilizing and lethal X-ray doses for significant developmental stages in the life-cycle of *Habrobracon juglandis* (Ashmead)

treatment and pre-eclosion, usually at some developmental crisis.

Specific controls were not used. Stock performance during the experiments and its subsequent performance are sufficient evidence of an unaltered and high reproductive capacity of untreated wasps.

The pattern of radiation-induced effects is shown in Fig. 1 for most of the life-cycle to demonstrate the divergence between sterilizing and lethal doses. At stages earlier than shown, the gonad is no more radiosensitive than the soma, since females developing after doses at the lethal threshold are as fertile as unexposed wasps. Very little radiation, 100–200 r., kills 1–3 hr. embryos. This, the weakest link in the life-cycle, is the time at which primitive germ cells differentiate and begin posterior migration. These cells are not seriously influenced by irradiation up to the dose that prohibits hatching. Selective irradiation of the posterior pole was not successful in destroying this cell population. The bulk of the egg (9–13 hr. old) was shielded with lead so that only the posterior pole was irradiated with 170 r./min. Samples were exposed from 1 to 90 min. The 10-min. exposure appeared to be lethal.

The curve (Fig. 1) for increased radiosensitivity does not progress smoothly. Biological considerations are offered in explanation. During early larval life, all basic organ systems differentiate from embryonic germ layers, but the business of ingestion seems of chief concern during the instars. Then at 90 hr. there is a highly active physiological condition in preparation for spinning, as well as critical internal histological changes. Then, somatic radioresistance increases appreciably at the postmeconium stage (114 hr.), followed by a drop back toward sensitivity in white pupæ. Compared with older forms which withstand up to 300,000 r. (ref. 2), white pupæ are very sensitive probably because muscle and other tissues are forming while the fat body, the predominant tissue, is resorbed. Active cell division and physiology could account for the radiosensitivity. Thus, the over-all pattern of effects appears consistent with a modernized version of the radiobiological 'law' of Bergonie and Tribondeau (see ref. 4) which stated that sensitivity of cells to radiation depends upon their reproductive activity and degree of differentiation.

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Changes in Structure of a Periclinal Chromosomal Chimera of Apple following X-Irradiation

THREE types of periclinal chromosomal chimeras in apple have been found in Nature[1–3]. Their apical meristems are characterized by one, two or three layers of diploid cells over tetraploid interior cells. Plants with one diploid layer (designated 2–4–4–4) produce diploid gametes, but the others (2–2–4–4 and 2–2–2–4) produce haploid gametes[2]. Since 2–4–4–4 chimeras are a source of diploid gametes for use in breeding polyploid apples, it would be desirable to produce this chimera in varieties in which only the other types have been found. X-rays have been shown to alter the structure of a periclinal chimera in carnation[4]. The effects of X-radiation on a 2–2–4–4 chimera of apple, 'Giant Spy (Loop)'[1–3,5], has been reported as an abstract[6] and are here described in more detail.

Dormant one-year-old shoots from trees seven to twenty years old of 'Giant Spy (Loop)' apple were irradiated in February, 1957, at Brookhaven National Laboratory, Upton, New York. A General Electric Maxitron X-ray machine was operated at 250 kVp. and 30 m.amp. with 1 mm. aluminium filtration to deliver doses of 3,000 r. A single layer of scions was exposed at one time and only those buds which occurred on the exposed side of the shoot were selected to grow. Treated and control scions were bench-grafted to apple seedlings and the plants forced in the greenhouse.

Buds just starting to grow were fixed in Randolph's modification of Navashin's fluid[7]. Slides of about 40 buds were prepared and stained in safranin and fast green[7] or in crystal violet[1].

The remaining plants were grown for two years. All trees sampled had grown from the irradiated scion and not from the rootstock. The terminal portion of each main branch (1–5 per tree) of 38 treated and 6 control trees was cut in the spring of 1959 and forced in water in the greenhouse. About 270 growing buds were collected and processed as described by Blaser and Einset[1].

The distribution of diploid and tetraploid cells, which were distinguished by the comparative size of division figures, chiefly metaphase plates[1], was noted

in the apical meristem, deeper stem tissues and young leaves of 2–15 buds per tree. Shoot tips with the constitution 2–2–4–4 and 2–2–2–4 could be distinguished only by comparison of figures in the apical meristem itself. At least one shoot tip with a definite determination of ploidy of layers was necessary to identify the type of chimera present.

Buds from the trees which were the source of the irradiated material were found to be 2–2–4–4 chimeras either in the present or earlier[2] studies. The chromosomal constitution of buds from control plants was 2–2–4–4.

Buds collected two weeks after forcing of the irradiated bench-grafted scions showed a pattern of radiation damage similar to that found in other irradiated apple shoot apices[7]. The walls, cytoplasm and nucleus of cells of the first layer generally stained like those of normal meristematic cells. Occasionally a few cells showed collapse or 'absence' of the protoplast. Many deeper cells in the central area of the tunica and corpus showed heavily stained walls, collapsed cytoplasm and disorganized nucleus, or protoplast so lightly stained that the cell appeared 'empty'. The axillary meristems had a pattern of radiation damage similar to that of the apical meristem.

In about one-third of the apices collected 2–6 weeks after forcing where the damaged area was still conspicuous, division figures were found in living cells near the damaged cells, both in the first layer and, more frequently, in the deeper cells.

The chromosomal constitution of branched or unbranched shoots which had grown for two years after irradiation of the original bud is presented in Table 1. The high frequency (33 out of 41 surviving buds) of types different from the one irradiated makes it probable that these have occurred as a result of shifting of layers during the re-organization of the shoot apex after radiation damage, and not as a result of changes in ploidy induced by radiation. Divisions observed in cells near the area of damaged cells suggest that a shoot apex may be formed from cells peripheral to the injury, either in the terminal or axillary meristems. Unfortunately, no stages in the re-organization of the apex were found, perhaps due to too widely spaced collections. The formation of a shoot primarily from the inner tetraploid cells with the original surface cells propagating themselves as a single diploid layer, which produces a 2–4–4–4 type, was much less frequent than formation of a new shoot from the upper diploid layers, which produces 2–2–2–4 and diploid shoots.

Table 1. Chromosomal Constitution of Two-year-old Shoots each from One Irradiated Bud of 'Giant Spy (Loop)' (2–2–4–4) Figures refer to the number of shoots of each type.

2–4–4–4	2–2–4–4	2–2–2–4	2–2–2–2	2–2–4–4 and 2–2–2–4	2–2–4–4 and 2–2–2–2	2–2–2–4 and 2–2–2–2
3	8	10	12	3	1	4

Eight shoots were not uniform in type (Table 1). The chromosomal constitution of buds in four instances was consistent within a branch, but in four others variable within a branch. The buds themselves appeared to be of one type. This lack of uniformity may result from the segregation of chimeral or diploid buds from different sectors of some reorganized apices. The instability of 2–2–2–4 chimeras may also be related to the slight tendency of natural 2–2–2–4 chimeras to produce diploid buds[3].

The pattern of radiation sensitivity of cells in the apple shoot apex, as indicated by their survival, is similar whether a wholly diploid shoot or a chimeral one is irradiated with a dose semi-lethal to the bud[7]. Sensitivity to these doses seems to be related to the position of the cells in the meristem rather than to the ploidy of those cells.

X-irradiation seems to be a tool for producing from 2–2–4–4 chimeras, which produce haploid gametes, 2–4–4–4 chimeras, which may be expected, barring chromosomal or genic changes, to produce diploid gametes for use in breeding polyploid apples.

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BIOLOGY

Autocatalytic Particles and Steady States

There are several phenomena in microbial heredity which are suggestive of cytoplasmic inheritance in that a particular self-perpetuating state can be induced rapidly in a large majority of the population by relatively simple changes in the growth conditions. These include long-term adaptation in yeast[1], auto-adaptation in bacteria[2], and the adaptive formation of β-galactosidose in *Escherichia coli* grown at maintenance-levels of inducer[3].

The first of these systems to be analysed in detail with respect to the points of interest here was long-term adaptation. One question which was asked was whether the ability of the cells of slow fermenters to metabolize galactose depends on the presence in these cells of some kind of autocatalytic particles. From an analysis of the kinetics of the loss during growth on galactose-free medium of the capacity to form galactose-positive colonies, it was deduced that this was indeed the case, and that a small number of such particles (of the order of one per cell) was necessary to function autocatalytically[4].

Several models were proposed for the mechanism of the autocatalysis, some of which did, and others of which did not, require that the particles be self-duplicating[5,6]. These have all been superseded by the recent detailed analysis of the β-galactosidose system of *Escherichia coli*[3,7], in which it was possible to equate the autocatalytic particle with the β-galactosidose permease.

There is one point in the discussion of these results which should be clarified. The 'autocatalytic particle' hypothesis originally considered for long-term adaptation is of course logically valueless unless one can imagine some alternative model.[8] The alternative generally considered is an aparticulate model depending on the existence of multiple steady states. There has been some disagreement in recent years[8] as to whether these two alternatives are really distinct. It has been implied that the 'steady state' hypothesis is simply a mathematical description which can be applied to autocatalytic particles among other

things[9]. This is presumably also the point of view of Cohn and Horibata[7], since they interpret their results, which certainly involve autocatalytic particles, to be an example of the steady-state model.

I wish to point out here : (1) that there is a logical distinction between autocatalytic particle models and stable steady-state models ; and (2) that no example of the latter has as yet been provided.

Steady states. Consider a cell which contains n chemical species involved in related reactions. Let c be the concentration of the ith species. Then we have, in general, a system of n simultaneous differential equations of the form :

$$\frac{dc_i}{t} = f_i\,(c_1\ c_2\ \cdots\ c_n)$$

A stable steady state is said to exist at some set of values $(C_1, C_2 \cdots C_n)$ of the concentrations if two conditions are satisfied : (1) $f_i\ (C_1, C_2 \ldots C_n) = 0$ for all i ; (2) the system will tend to return to the steady state following a sufficiently small displacement in any direction from the steady-state values.

It is easy to find examples of such steady state. It is also easy to construct examples in which more than one solution exists for condition (1). Condition (2) is also easy to satisfy for a single solution ; but it is not satisfied by most of the systems with multiple solutions which have been proposed in more or less mathematical detail by various authors[10,11].

Condition (2) is, however, critical. Without it, the steady state is unstable in the same sense that the equilibrium of a cone balanced on its vertex is unstable. Any small fluctuation will cause it to diverge permanently from the steady state in question.

Autocatalytic particles and unstable steady states. The autocatalytic particle hypothesis can be defined as follows. There is some subcellular particle X with the properties that : (*a*) A cell with at least one particle of X will synthesize more such particles during growth ; (*b*) a cell with no such particles will not form any.

Such a system will generally have at least two steady states. One of these (at $X = 0$) is, however, unstable. The most basic distinction is therefore between stable and unstable steady states, the autocatalytic particle hypothesis being a special case of the latter. The mechanism of autocatalysis is irrelevant in the context.

Condition (*b*) cannot of course be taken literally, since any molecular event must have some non-zero probability. In practice, what is meant is that the probability of forming a particle during one cell division cycle is much less than one. The genes themselves approximate this rule well enough to have allowed the construction of the science of genetics.

Stable steady states could in principle be maintained indefinitely in growing, well-mixed tubs of protoplasm which are never subdivided into smaller isolated regions. The 'hereditary' character of unstable steady states, on the other hand, depends completely on the fact that the cell has a finite size. Each cell division creates a certain possibility for the occurrence of occasional cells with a large disparity in the numbers or kinds of certain critical molecules, and one such fluctuation has occurred, its results being confined to the progeny of the cell in which it took place. The process is reminiscent of genetic drift or gene fixation ; or, more properly, these phenomena are themselves examples of unstable steady states within a population rather than within a cell.

Another type of unstable state may explain some of the antigenic changes in *Paramecium*, for which Delbrück's[10] steady-state hypothesis was originally proposed. These are states which are not steady at all, but for which the change from one state to another is sufficiently slow to require many cell generations. The continuation of such states for a limited number of generations does not depend on finite cell size, and they cannot be perpetuated indefinitely even among sublines continually selected as being apparently in the original state.

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Chromosome Numbers of Slugs

CHROMOSOME numbers are becoming increasingly part of the taxonomic descriptions of animal and plant species.

Very few figures are known for the slugs, the collective name given to members of the three families of almost shell-less pulmonate molluscs which occur in Britain. Some pulmonate species have been examined in France and Switzerland by M. and J. L. Perrot[1-3], and the comparison of their results with mine, given in Table 1, shows that the number of chromosomes is the same for British and Continental samples. No evidence has been found of chromosome races, as in the populations of *Sorex araneus* studied by Ford *et al.*[4].

Chromosome counts were made at the first meiotic prophase and metaphase stages of spermatogenesis, and also the mitotic metaphases found in ovotestes of mature or nearly mature animals. The ovotestis was fixed in alcohol-acetic, teased to separate the cells, and stained and squashed in aceto-orcein.

The taxonomic arrangement of this list is that of Quick[5], containing the alteration of *Limax arborum* Bouchard-Chantereaux to *Lehmannia marginata* Muller ; the name *Lehmannia* having been used generically by Malm in 1868. Cytotaxonomy fully supports the withdrawal of this species from the genus *Limax* ; a change made on the structure of the radula. *Limax tenellus* seems to correspond more closely with *Lehmannia marginata* in chromosome number than with the other three British species of *Limax*. Intrageneric variation in chromosome number in the genus *Arion* emphasizes the close relationship between *A. ater* L. and *A. rufus* L. ; considered as subspecies by Cain and Williamson[6].

Little or no variation has been found within the other genera, and the number of chromosomes cannot

Table 1

Family	Genus	Subgenus	Species	Author	Chromosome number	
					Present work	Counts by Perrot and Perrot
Testacellidae	*Testacella*		*haliotidea*	Draparnaud	$n = 32$	—
Arionidae	*Arion*	*Micrarion*	*intermedius*	Normand	$n = 28$	—
		Carinarion	*circumscriptus*	Johnston	$n = 29$	—
		Kobeltia	*hortensis*	Ferussac	$n = 28$	—
		Mesarion	*subfuscus*	(Draparnaud)	$n = 25$	—
		Arion s.s.	*ater*	(L.)	$n = 26$	—
			*rufus**	(L.)	$n = 26$	
Limacidae	*Milax*		*gagates*	(Draparnaud)	$n = 33$ or 34	—
			sowerbii	(Ferussac)	$n = 34$	—
			gracilis	(Leydig)	$n = 33$	—
	Limax	*Malacolimax*	*tenellus*	Nilsson	—	$n = 24$
		Limax s.s.	*cinereoniger*	Wolf	$n = 31$	$n = 31$
			maximus	(L.)	$n = 31$	$n = 31$
		Limacus	*flavus*	(L.)	$n = 31$	$n = 31$
	Lehmannia		*marginata*	Muller	$n = 24$	$n = 24$
	Agriolimax	*Agriolimax*	s.s. *agrestis*	(L.)	$n = 30$	—
			reticulatus	(Muller)	$n = 30$	†
		Malinx	*laevis*	(Muller)	$n = 30$	—
			caruanae	Pollonera	$n = 30$	—

* Collected in France.
† Perrot, J. L., quotes $n = 30$ for *A. agrestis*, but probably refers to animals now included in *A. reticulatus*.

be used to distinguish between morphologically rather similar species of, for example, *Agriolimax*. *Testacella maugei* Ferussac, *T. scutulum* Sowerby, and *Geomalacus maculosus* Allman, on the British list, are not included in Table 1, as no cytological analysis has been carried out.

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An Apparatus for Panning in Cine Micrography

In ciné micrography it has not been possible to vary the viewpoint or introduce variety by devices equivalent to 'panning' or 'tracking' in ordinary ciné photography. This limitation makes it difficult to show some aspects of a microscopical field which demand the use of a moving stage.

The movement of the usual rack and pinion of the mechanical stage of a microscope, when enlarged 300–400 times on the film before projection, is so irregular owing to the backlash and the tremor of the operator's hand and the need for changing the grip that it is quite unacceptable.

After a number of attempts, a successful apparatus has been made which combines the principles used in the De Fonbrune and the Singer micromanipulators. This gives smooth control of movement in both directions separately or combined.

The arrangement consists of two pieces of aluminium plate, the bottom one bolted by two easily detachable brass brackets to the microscope stage, the upper one stuck to it with petroleum jelly.

The lower plate has a central hole over the condenser lens, and the upper plate has an excentric opening which overlaps the condenser hole by about 2 cm. When arranged ready for use, the upper plate projects for about 2 cm. beyond the lower, both to the left and forward.

Two small syringes with metal plungers are mounted horizontally and at right angles to each other beside the stage. Slots cut in the heads of the plungers rest against the upper, moving plate. This gives both a certain grip and at the same time permits automatic re-arrangement of one syringe as the other is in use. Both syringes are carried at the correct height by metal rods on top of which are spring clips. The rod which provides anterior–posterior movement is placed off-centre to clear the beam of light from the lamp. A plastic or wooden base with stops for the foot of the stand is also required. The supporting rods are screwed to this.

Each of the syringes is connected to another syringe by narrow rubber pressure tubing. Plastic tubing is too rigid in the wall and too springy to be a satisfactory alternative. The syringes on the free end of the rubber tubes are pressed to drive the stage. They need not be the same size as the others, but no advantage appeared to be derived from using smaller syringes as no gearing ratio was necessary in practice.

In use, the slide to be filmed is placed on the upper sliding plate. The pair of syringes on the free ends of the tubing are held between the fingers of the left hand and their plungers compressed with the thumb; one alone, or both, may be used to give movements in two directions at right angles or diagonal movement. The right hand is available for the normal controls of the microscope.

No special return mechanism is provided. At the end of the run, the sliding plate is simply pushed back to the starting position.

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GENETICS

Position of the Gene determining the Diploid-like Meiotic Behaviour of Wheat

Common wheat (*Triticum aestivum* $2n = 6x = 42$) has attained the hexaploid condition by the combination of the full chromosome complements of the three diploid species *Triticum monococcum*, *Aegilops speltoides* and *Aegilops squarrosa*[1-3]. However, at meiosis in *T. aestivum*, despite the close genetical and cytological relationships of its diploid ancestors, each chromosome pairs with its fully homologous partner and only bivalents are formed. There is no pairing between corresponding, homœologous, chromosomes derived from different diploid ancestors, although considerable similarity in the genetical activities of homœologues still remains[4].

Riley and Chapman[5] showed that homœologous pairing was no longer precluded in the absence of a particular chromosome, the *HH* chromosome, from 20-chromosome haploids and 40-chromosome nullisomics of the variety Holdfast. Thus the control of the diploid-like, bivalent-forming, meiotic regime in wheat can be ascribed to a gene-system on this chromosome.

Simultaneously, Sears and Okamoto[6] interpreted the high incidence of meiotic pairing in hybrids between *T. monococcum* and *T. aestivum* variety Chinese Spring, deficient for chromosome V, to mean that the regular meiotic behaviour of wheat was due to the activity of this chromosome.

The deficiency of the *HH* chromosome in nullisomics of Holdfast, and of chromosome V in nullisomics of Chinese Spring, results in equivalent modifications of external plant morphology. Also, both chromosomes are alike in structure, being among the largest in their respective complements, and having one arm more than twice the length of the other. Thus it seems likely that they are homologous, but for an understanding of the cytogenetic structure of wheat it is necessary to determine their exact relationships.

For this purpose hybrids were made between 41-chromosome plants of Holdfast, with the *HH* chromosome monosomic, and plants of Chinese Spring in which chromosome V was represented by a pair of telocentrics for the long arm and the other arm was completely deficient. At meiosis, the 41-chromosome hybrids, which had not received the unpaired *HH* chromosome from Holdfast, had 20 normal bivalents, and the telocentric of chromosome V, from Chinese Spring, was a univalent. Consequently, V and *HH* must be fully homologous chromosomes and investigation of the two varieties concerned has revealed the same genetic control of meiosis.

Since the absence of chromosome V results in homœologous pairing, there is evidently no independent control, on any other chromosome, capable of causing the meiotic isolation of chromosomes of the different basic diploid sets. However, it might have been that the gene on chromosome V was part of a complementary system, the removal of any part of which would result in the breakdown of the normal regulation of meiosis. To test this a series of 21 wheat – rye hybrids was made in which hybrids were deficient, in turn, for each of the 21 chromosomes of wheat. The hybrids were produced by pollinating with rye (*Secale cereale* $2n = 14$) the 21 lines of Chinese Spring wheat which were monosomic for chromosomes I–XXI. Use was made of the 27-chromosome hybrids to which the chromosome which was unpaired in the monosomic wheat parent had not been transmitted.

Table 1. Mean Chromosome Pairing at First Metaphase of Meiosis in Normal Hybrids of *T. aestivum* × *S. cereale* and in the 21 Hybrids Deficient, in Turn, for each Wheat Chromosome

Chromosome number	Chromosome deficient	Cells scored	Bivalents	Trivalents	Quad.
28	—	300	0·48 ± 0·0435	—	—
27	I	100	0·11 ± 0·0386	—	—
27	II	100	0·15 ± 0·0352	—	—
27	III	100	0·14 ± 0·0446	—	—
27	IV	100	0·37 ± 0·0684	0·01	—
27	V	200	3·36 ± 0·1591	0·88 ± 0·0326	0·08
27	VI	100	0·31 ± 0·0581	—	—
27	VII	100	0·40 ± 0·0685	—	—
27	VIII	100	0·32 ± 0·0584	0·01	—
27	IX	100	0·75 ± 0·0936	—	—
27	X	100	0·33 ± 0·0587	0·01	—
27	XI	100	0·06 ± 0·0239	—	—
27	XII	100	0·49 ± 0·0595	—	—
27	XIII	100	0·12 ± 0·0331	—	—
27	XIV	100	0·22 ± 0·0504	—	—
27	XV	100	0·71 ± 0·0808	—	—
27	XVI	100	1·00 ± 0·0885	0·04	—
27	XVII	100	0·77 ± 0·0925	—	—
27	XVIII	100	0·86 ± 0·0363	—	—
27	XIX	100	0·47 ± 0·0666	—	—
27	XX	100	0·23 ± 0·0423	—	—
27	XXI	100	0·29 ± 0·0478	—	—

Riley, Chapman and Kimber[7] have already shown that there is much higher pairing in wheat – rye hybrids, deficient in chromosome V, than in normal, euploid hybrids. The extra pairing results from homœologous associations within the wheat complement as well as from associations of wheat and rye chromosomes. It may, therefore, be expected that the absence from a wheat – rye hybrid of any other wheat chromosome, carrying a gene similar in effect to that on chromosome V, would cause similarly altered meiotic pairing.

First metaphase of meiosis was examined in 50 pollen mother cells from each of at least two plants of every type of chromosome-deficient hybrid, as well as in six euploid hybrid controls (Table 1). Hybrids deficient for chromosome V had much higher pairing than euploid hybrids, and higher pairing than hybrids deficient for any other chromosome. The mean number of trivalents in V-deficient hybrids exceeded the mean number of bivalents in most other lines, and the number of bivalents was more than three times greater in the absence of V than in any other condition. There can be no doubt that the deficiency of chromosome V is unique in its effect on meiotic behaviour, for the differences among all the other hybrids are of an order which may be ascribed to environmental effects.

Thus the meiotic isolation of corresponding chromosomes of the different genomes of wheat is due to the activity of chromosome V alone. It has also been shown that this activity is due to the long arm of the chromosome[8], but there is no evidence concerning the number of loci involved.

However, the localization is consistent with the notion that a single mutant gene, having the observed effect on meiosis, was selected because of its influence on fertility and genetic stability, early in the development of polyploidy in *Triticum*. The selection of a single powerful, cytologically stabilizing, mutant would presumably be rapid and efficient. By contrast, it is difficult to visualize the processes by which, as required by the discarded alternative hypothesis, a system of complementary genes controlling the diploid-like meiosis could have evolved. This would

have required the independent selective incorporation of a number of mutually dependent genes, each of which was vital for the occurrence of regular meiotic behaviour with its attendant reproductive stability.

Nevertheless, genes other than the one at the critical locus on chromosome V must influence the meiotic behaviour of wheat, and some may modify in one direction or another the regular, bivalent-forming, pattern. Meiosis in wheat is, therefore, delicately balanced between excessive pairing leading to multivalent formation and genetic instability, and failure of pairing, leading to univalent formation and aneuploidy. This balance has made possible the simultaneous occurrence of pronounced genetical triplication with high fertility and stable inheritance.

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Experimentally Induced Inter-bivalent Redistribution of Chiasmata in *Delphinium*

The non-random distribution of chiasmata in a bivalent due to the occurrence of interference is well known. It has been suggested by some authors that the inter-bivalent distribution of chiasmata may also not be independently determined. Correlation studies on chiasma frequencies have demonstrated competition for chiasmata between bivalents in some organisms but not in others[1,2]. In the course of investigations on the cytogenetic effects of ionizing radiations on plants, we have been able to affect changes of chiasma frequency in one group of chromosomes in the nucleus and observe the reaction of the other group to this experimentally induced condition.

Dry seeds of *Delphinium ajacis* variety 'Comet' were irradiated with an X-ray dose of 15,000 r. (50 kV.). The X_1 plants raised from these showed certain cytological characteristics which appeared to be of interest. Apart from gross structural changes in the chromosomes of some of the plants which indicate interchange multiple associations, several others were characterized by disturbed chromosome pairing and chiasma frequencies. Observations on the control plants show that eight bivalents are regularly formed in most of their pollen mother-cells, a few of the cells showing seven pairs and two univalents. Since two of the eight chromosome pairs are considerably larger than others, they can be easily identified, and they appear mostly as ring bivalents at metaphase with two terminal or sub-terminal chiasmata; the remaining six smaller pairs appear in most pollen mother-cells as one ring, and five rods or all rods, each with a sub-terminal or nearly terminal chiasma. The two univalents whenever present are members of one of the smaller pairs of chromosomes.

The plants from the seeds treated with radiation differ from the controls in having two or more univalents present in many of their pollen mother-cells. Both the large and the small group chromosomes may appear as univalents, quite often in the same pollen mother-cell; their number varies from plant to plant as shown in Table 1. Also shown are observations on each of the 5 control and 9 X_1 plants with regard to the mean number of ring bivalents found in their pollen mother-cells. The proportion of rings formed by the large and small chromosomes respectively has been indicated separately.

The results for X_1 plants have been arranged in an order which helps to emphasize the relationship that appears to exist between the frequencies of the large and the small ring bivalents. The results show clearly that as the frequency of ring bivalents formed by the large chromosomes is reduced due to their occurrence as univalents or rod bivalents, the frequency of such configurations formed by the smaller chromosomes is either increased significantly beyond the level in the control material, as in plants 4, 6 and 11, or is maintained at a similar level, as in plants 7, 8 and 9, in spite of the fact that a number of small chromosomes also appear as univalents. In some of these plants it is quite common to find pollen mother-cells in which the two large ring bivalents were replaced by a similar number of small ring bivalents. The only exception is provided by plant 12, in which the frequencies of both the large and the small rings are reduced.

Although both chromosome pairing as well as chiasma formation and terminalization are known to be genotypically controlled, it is obvious that neither

Table 1. Mean Frequencies of Univalents and Ring Bivalents in the Control and X_1 Plants

Plant No.	No. of pollen mother-cells	Frequency of univalents: Mean No. of univalents per cell	Frequency of univalents: Percentage of small univalents	Mean percentage chromosome pairs as: Large ring bivalents	Mean percentage chromosome pairs as: Small ring bivalents	
Controls (*C*)						
1	20	—	—	20·00	9·38	
2	25	—	—	22·00	9·50	
3	25	—	—	21·50	6·50	$\overline{C} = 7{\cdot}48 \pm 0{\cdot}955$
4	25	—	—	23·50	5·50	
5	25	—	—	23·50	6·50	
Treated (*T*)						
4	25	10·48	67·18	1·50	17·00	
11	18	3·33	60·00	5·50	11·75	$\overline{T} = 14{\cdot}25 \pm 1{\cdot}234$
6	25	2·40	16·67	10·50	14·00	
12	14	3·14	77·23	10·71	3·57	
9	24	2·00	100·00	14·49	7·99	
8	20	2·00	100·00	15·62	7·50	
7	14	2·00	100·00	17·00	5·37	
13	15	2·40	94·45	20·00	3·25	
10	20	2·00	100·00	20·00	1·25	

$$t = \frac{\overline{T} - \overline{C} = D}{\text{S.E.}(D)} = \frac{6{\cdot}77}{1{\cdot}56} = 4{\cdot}34^{**}$$

the occurrence of univalents nor the changes in chiasma frequency in the plants from X-irradiated seeds are genetically determined. Not only do the univalents occur in most of the X_1 plants but also their frequency shows great variation; and the smaller chromosomes, which outnumber the larger ones, are affected more often as would be expected on a random basis. It may be supposed that the X-irradiation of seeds induces structural changes in a number of chromosomes at random, affecting their pairing at pachytene and formation of chiasmata. The re-distribution of chiasmata described above occurs probably in those plants in which a number of smaller chromosomes have escaped damage.

The non-random distribution of chiasmata, for which evidence has been presented here, emphasizes the co-ordination existing in the control of chiasma formation in different chromosomes in the nucleus. How this control is exercised is not very evident. The chromosome changes induced by radiation, which result in the re-distribution of chiasmata, also stress the evolutionary significance of spontaneously occurring structural changes of chromosomes. Not only may many of these changes give rise to new variation by producing effects which simulate point mutations, but also they may release latent variability through re-distribution of points of crossing-over. The importance of such re-distribution resulting from a different kind of agency, in relation to natural selection, has been discussed by Mather and Harrison[4].

We are grateful to Dr. B. P. Pal and Dr. A. B. Joshi for their interest and encouragement. Thanks are also due to Dr. M. S. Swaminathan for discussing the results with us.

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VIROLOGY

Somatic Antigen 15 as a Precursor of Antigen 34 in *Salmonella*

Phage ε^{34} is a temperate phage produced by group E_3 *Salmonellae* with O antigens (3), (15), 34 and capable of converting group E_3 *Salmonella* (O antigens 3, 15) to group E_3 *Salmonella* (O antigens (3), (15), 34) by lysogenization[1–5]. The formation of antigen 34 is accompanied by partial loss of antigens 3 and 15[5], suggesting a close relationship between antigens 34 and 3 or 15. The experiments described here indicate that antigen 15 is a precursor of antigen 34.

Group E_1 *Salmonellae* with O antigens 3, 10 do not adsorb phage ε^{34}. However, the possibility of infecting group E_1 cells directly with phage ε^{34} is suggested by the experiments of Uetake, Luria and Burrous[6]. They reported that when *Salmonella anatum* (group E_1, O antigens 3, 10) is infected with phage ε^{15}, antigen 15 and receptors for phage ε^{34} appear within a few minutes, the cells which survive infection segregate phage-carrier and non-carrier cells in their progeny, and in the non-carrier segregants the changes in antigenic structure and in phage receptors initiated by phage infection persist for several generations. Therefore, if the non-carrier progenies with receptors for ε^{34} are infected with ε^{34}, *S. anatum* might probably be lysogenized with ε^{34} without carrying ε^{15}. This proved true in the following experiments.

Cells of *S. anatum* were infected with phage ε^{15} at a multiplicity of 3·6, and progenies of the cells, which survived infection with ε^{15}, were infected with another phage ε^{34} 3 hr. after ε^{15}-infection, at which time about 90 per cent of cells were not carrying ε^{15}. Approximately 87 per cent of cells survived infection with ε^{34}. Among the survivors, 82 per cent proved to produce ε^{34}, but most of them not to produce ε^{15} by replica plating[6,7]. By agglutination and absorption tests these ε^{34}-producing cells ($= A(\varepsilon^{34})$) proved to possess somatic antigen 3, 10, the same structure as that of *S. anatum*. Most important is that they do not possess antigen 34. They are sensitive to phage ε^{15} and its mutants, while they adsorb neither phage ε^{34} nor its mutants, being similar to *S. anatum*. The only difference from *S. anatum* is in resistance to phage C_{341} to which *S. anatum* is sensitive, although $A(\varepsilon^{34})$ cells still adsorb phage C_{341} at an adsorption-rate constant of $3{\cdot}1 \times 10^{-9}$ ml. per min. (Table 1). When $A(\varepsilon^{34})$ cells were infected with ε^{15}, both antigens 15 and 34 appeared within several minutes in infected cells.

Among the above, most remarkable are the findings that $A(\varepsilon^{34})$ cells do not form antigen 34 irrespective of presence of prophage ε^{34}, which always leads to the formation of antigen 34 when it infects 3, 15 cells of group E_2, and that antigen 34 appears when $A(\varepsilon^{34})$ cells form antigen 15 by infection with phage ε^{15}. These observations, together with Nakagawa's finding[5] mentioned above, indicate that the formation of antigen 15, at least partially, is a necessary prerequisite for the formation of antigen 34. Antigen 3 is also partially lost when 3, 15 cells are converted to (3), (15), 34 by lysogenization with ε^{34}; but it does not seem to be necessary for the formation of antigen

Table 1

Bacterial strain	Sensitivity to phage (/ml.)												O antigens
	ε^{15}			ε^{sv}			C_{341}			ε^{34vir}			
	10^{10}	10^{7}	10^{4}	10^{10}	10^{7}	10^{4}	10^{10}	10^{7}	10^{4}	10^{10}	10^{7}	10^{4}	
$A(\varepsilon^{34})$	+	+	+	+	+	+	(+)	−	−	−	−	−	3, 10
A	+	+	+	+	+	+	+	+	+	−	−	−	3, 10
$A(\varepsilon^{15})$	−	−	−	−	−	−	−	−	−	+	+	+	3, 15
$A(\varepsilon^{15}, \varepsilon^{34})$	−	−	−	−	−	−	−	−	−	−	−	−	(3), (15), 34

Phage sensitivity was examined by spot test. ε^{sv} is a mutant of ε^{15}. As to the phages C_{341} and ε^{34vir}, refer to Uetake *et al.* (ref. 6). +, lysis; −, no lysis; (+), abnormal lysis.

34, since antigen 3 of *S. anatum* is identical with that of group E_2 cells or of $A(\varepsilon^{15})$ cells but there is neither formation of antigen 34 nor loss of antigen 3 in $A(\varepsilon^{34})$.

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Virus Release from Single Cells of Mouse Parotid Tumours

In vitro studies have shown that cells from polyoma-induced mouse tumours in mass culture can release virus, and that the release can vary in different tumours[1,2]. One of the factors responsible for this difference in release is the proportion of virus-releasing cells as measured by plating the tumour cells on mouse embryo monolayers, and determining the relationship between the number of tumour cells plated and the number of plaques[3]. The present communication is concerned with a study on single cells from mouse parotid tumours grown in microdrops, in order to obtain information on: (*a*) the proportion of cells releasing virus by plating in microdrops, and (*b*) the virus release per cell.

The parotid tumours were induced by inoculation of polyoma virus into 2- or 3-day old mice, and the tumour cells were dispersed by trypsinization with a 0·25 per cent trypsin solution (trypsin 1–300). Virus release per cell was tested either by seeding the cells directly in microdrops, or by seeding in microdrops cells that had been in mass culture. In all cases, the medium consisted of 0·5 per cent lactalbumin hydrolysate in Earle's saline and 20 per cent horse serum.

Cell suspensions were diluted in medium to about 10^3 cells in a 50-mm. Petri dish, and these were then used for the single cell seedings. The single cells were distributed freehand in microdrops with micropipettes under a dissecting microscope in an enclosed box, maintained at 37° C., with a humidified atmosphere containing 10 per cent carbon dioxide. The microdrops were made in 50-mm. Petri dishes under silicone fluid (Dow 'Corning 200')[3], and the Petri dishes with microdrops were kept in an incubator with a humidified atmosphere containing 10 per cent carbon dioxide. The presence of only a single cell in each microdrop was checked by observation with an inverted phase-contrast microscope.

For collecting the samples, each drop was sucked up with a micropipette, and the cell was then washed twice with new medium. The complete sample per cell thus consisted of three drops, which were added to 0·2 ml. of medium. The samples did not include cell-associated virus. When cells were tested after they had been in mass culture (5×10^6 cells seeded in a 50-mm. Petri dish) the cultures were washed twice with medium and the cells removed from the glass by trypsinization with a 0·25 per cent trypsin solution for 10 min. at 37° C. Not all the cells came off the glass by this procedure, and only those that were detached after 10 min. trypsinization were used for the microdrops. The samples collected from the single cells were tested for plaque-forming units on mouse embryo monolayers as described previously[4]. The number of plaque-forming units was read at 11 days after inoculation of the monolayers, and in the case of *MP*12 again at 16 days.

The proportion of single cells, from 6 parotid tumours, releasing virus under these conditions is shown in Table 1. Cells from two of these tumours were tested without previous mass culture, and the samples were taken at periods of 28–80 hr. after seeding the single cells. The highest proportion of releasing cells, 9/19, was found in tumour *MP*2, and this tumour had also given the highest release by cells in mass culture (hæmagglutination titre of 2,048 per ml. on the day equivalent to that used for sampling from microdrops). In the other five tumours, virus release was detected in only 3 out of 172 cells tested. None of the single tumour cells was seen to divide under these conditions.

Table 1. Proportion of Single Cells from Six Mouse Parotid Tumours releasing Virus in Microdrops

Tumour	Strain of mouse	No. of days in mass culture	No. of cells tested	No. of cells releasing virus	Release period (hr.)
*MP*4	*AKR*	0	16	1	72
*MP*3	*AKR*	0	13	0	80
*MP*2	*AKR*	2	19	9	28
*MP*12	*DBA*/2	3	26	1	28
*MP*13	*SWR*	4	76	1	36 and 72
*MP*14	*SWR*	4	41	0	36 and 72

Total No. of cells tested, 191; total No. of cells releasing virus, 12.

The number of plaque-forming units released per cell in tumour *MP*2 during a 28-hr. release period is shown in Table 2. The release varied from 0 to 9 plaque-forming units per cell with an average of 5 for the virus-releasing cells. This figure is similar to that reported for the Rous chicken sarcoma[5], and whether parotid tumour cells have a regular release per day is now being tested. The release from the other 3 positive cells (Table 1) in 3 other tumours were 17 plaque-forming units in *MP*4, 4 in *MP*12, and 2 in *MP*13.

Table 2. Virus Release from Single Cells in Microdrops during a 28-hr. Period

No. of plaque-forming units per cell	0	1	2	3	4	5	6	7	8	9
No. of cells	10	0	1	2	2	1	1	1	0	1

No. of cells releasing virus, 9/19; average release of virus releasing cells, 5 plaque-forming units.

The above results provide further information on the existence of a difference in virus release from different tumours, and they also show that under the conditions of these tests in microdrops, single cells from polyoma-induced parotid tumours have at most only a low virus release per day.

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FORTHCOMING EVENTS

Wednesday, April 20

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Annual General Meeting. Dr. J. M. Stagg: Presidential Address.

FACULTY OF THE HISTORY OF MEDICINE AND PHARMACY (at the Worshipful Society of Apothecaries of London, Black Friars Lane, London, E.C.4), at 5.30 p.m.—Sir John Charles, K.C.B.: "Roger Bacon on the Errors of Physicians" (The Gideon Delaune Lecture).

Wednesday, April 20—Thursday, April 21

SOCIETY OF CHEMICAL INDUSTRY, AGRICULTURE GROUP (in the Hoare Memorial Hall, Church House, Westminster, London, S.W.1), at 10 a.m. daily—Symposium on "Advances in the Chemical Analysis of Soils, Fertilizers and Plants".

Thursday, April 21

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. A. R. Gibson and Mr. J. R. Chalkley: "Production of Thorium Metal"; Prof. H. Borchert: "Genesis of Marine Sedimentary Iron Ores".

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.: Mr. R. I. Currie, Dr. D. H. Cuching and Dr. G. E. Fogg: "Productivity of the Sea".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Prof. E. Artin: "On a Theorem of Gelfand with Applications to Number Theory".

PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department, Imperial College of Science and Technology, Imperial Institute Road, London, S.W.7), at 5.30 p.m.—Annual General Meeting, followed by Mr. R. S. Forster: "Noise—Legal Control".

ROYAL AERONAUTICAL SOCIETY (at the Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. J. E. Allen: "On Reducing Costs of Space Research".

SOCIETY OF CHEMICAL INDUSTRY, CORROSION GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Annual General Meeting, followed by Mr. D. H. Collins: "Electrode Processes in Primary Batteries".

BRITISH INSTITUTION OF RADIO ENGINEERS, LONDON SECTION (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m. Dr. J. G. Nicholls: "Nerve Impulses from Stretch Receptors in Muscle".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Scientific Papers.

Thursday, April 21—Friday, April 22

FERTILIZER SOCIETY AND SOCIETY FOR ANALYTICAL CHEMISTRY (at Church House, Westminster, London, S.W.1), at 2.45 p.m. on Thursday and 9.15 a.m. on Friday—Symposium on "Fertiliser Analysis".

PLANT PHENOLICS GROUP (at Shoreditch College, Egham, Surrey)—Symposium on "Current Chemical Research on Plant Phenolics".

Friday, April 22

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.15 p.m.—Discussion of papers. Chairman: Dr. R. Stoneley, F.R.S.

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 5.15 p.m.—Mr. George L. Hansen and Mr. Richard W. James: "The Economics of Ship Routing".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Broadening University Courses", opened by Prof. H. E. M. Barlow.

ROYAL AERONAUTICAL SOCIETY, AGRICULTURAL AVIATION GROUP (at 4 Hamilton Place, London, W.1), at 7 p.m.—Meeting on "Pilot Training".

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting on "Unconventional Forms of Energy". Chairman: Prof. Pierre Auger. Speakers: Prof. S. Sambursky, Mr. P. D. Dunn and Prof. M. W. Thring.

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

DEPUTY LIBRARIAN (with a good honours degree and administrative experience in a library) OF THE BROTHERTON LIBRARY—The Registrar, The University, Leeds (April 21).

ASSISTANT EXPERIMENTAL OFFICER (with a degree in science or horticulture) IN THE ENTOMOLOGY SECTION; and an ASSISTANT EXPERIMENTAL OFFICER (with a degree or higher national certificate) IN THE CHEMISTRY SECTION—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (April 23).

ASSISTANT, Grade B (with a good honours degree and preferably some teaching experience) IN CHEMISTRY at Brighton Technical College, to teach general chemistry up to G.C.E. (Advanced Level) standard and to assist with the teaching of inorganic chemistry at degree level—The Director of Education, 54 Old Steine, Brighton (April 23).

EXPERIMENTAL OFFICER (with at least a pass degree or equivalent, and preferably considerable experience in a biochemical or pharmacological laboratory)—The Secretary, Institute of Animal Physiology, Babraham, Cambridge (April 23).

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Sheffield (April 25).

LECTURER (with a university degree in mechanical or chemical engineering or equivalent qualifications) IN CHEMICAL ENGINEERING—The Registrar, King's College (University of Durham), Newcastle upon Tyne 1 (April 25).

TECHNICAL ASSISTANT (with some knowledge of zoology), for culture work in protozoan research—Dr. E. M. Brown, Department of Zoology, Chelsea College of Science and Technology, Manresa Road, London, S.W.3 (April 25).

ASSISTANT ANALYST (member of the Royal Institute of Chemistry and practical experience in the examination of water samples)—The Public Analyst, City of Leicester Health Department, 7 Salisbury Road, Leicester (April 26).

SENIOR SCIENTIFIC OFFICER (with an honours degree in microbiology or in agricultural science, and postgraduate experience in food bacteriology, preferably in eggs and egg products) IN THE AGRICULTURAL BACTERIOLOGY DIVISION, Ministry of Agriculture—The Secretary, Civil Service Commission, Stormont, Belfast 4 (April 26).

SENIOR LECTURER or LECTURER IN PHILOSOPHY; and a SENIOR LECTURER or LECTURER IN AGRICULTURAL CHEMISTRY at the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 27).

ASSISTANT (biochemist, with a first- or second-class honours degree) IN BACTERIOLOGY at the Western Infirmary—The Secretary of University Court, The University, Glasgow (April 30).

ASSISTANT IN THE DEPARTMENT OF CHEMISTRY—The Secretary, The University, Edinburgh (April 30).

ASSISTANT INSPECTOR (HORTICULTURE) (man or woman at least 25 on May 1, 1960, with a degree or diploma in horticulture and at least five years appropriate practical experience, and able to drive a car)—Department of Agriculture and Fisheries for Scotland, Room 266, St. Andrew's House, Edinburgh 1 (April 30).

ASSISTANT LECTURER IN CROP PRODUCTION; and an ASSISTANT LECTURER IN ANIMAL PRODUCTION, with special reference to Dairy Production—The Registrar, Wye College (University of London), near Ashford, Kent (April 30).

LECTURER (with a degree in engineering and Corporate Member of the appropriate professional institution) IN ELECTRICAL ENGINEERING at the Royal Technical College of East Africa, Nairobi—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (April 30).

LECTURER (with a veterinary, medical or science degree) IN VETERINARY PHYSIOLOGY—The Secretary of University Court, The University, Glasgow (April 30).

LECTURER or ASSISTANT LECTURER IN MATHEMATICAL STATISTICS; and a LECTURER or ASSISTANT LECTURER IN APPLIED MATHEMATICS—The Secretary, The University, Exeter (April 30).

LECTURERS (3) IN PHARMACEUTICAL CHEMISTRY at the Nigerian College of Arts, Science and Technology, Ibadan—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (April 30).

STATISTICIAN (Scientific Officer or Senior Scientific Officer), to be responsible for advising on statistical aspects of (*a*) the design and interpretation of environmental surveys of radioactive substances in human diet and agricultural materials; and (*b*) a programme of laboratory and field experiments in soil science and plant physiology—The Secretary, Agricultural Research Council, Radiobiological Laboratory, Letcombe Regis, near Wantage, Berks (April 30).

ASSISTANT (medical, veterinary or science graduate with B.Sc. (Hons.) in pharmacology or physiology) IN THE DEPARTMENT OF PHARMACOLOGY—The Secretary, Department of Pharmacology, University College, Merville Foster Avenue, Stillorgan, Dublin (May 1).

SENIOR LECTURER (registered medical graduate) IN ANATOMY—The Secretary of University Court, The University, Glasgow (May 1).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF GEOLOGY (with special reference to Economic Geology and Geophysics)—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (May 7).

LECTURER or ASSISTANT LECTURER (with a degree in physiology or some related subject) IN PHYSIOLOGY; and a LECTURER or ASSISTANT LECTURER (with a degree in biochemistry or some related subject) IN BIOCHEMISTRY—The Registrar (Room 29, O.R.B.), The University, Reading (May 7).

SENIOR LECTURER or LECTURER (honours graduate in electrical engineering or physics and with suitable research and teaching experience in electronics and radio communication) IN ELECTRICAL ENGINEERING—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (May 7).

ASSISTANT LECTURER (with a good honours degree in physics and preferably some interest in electronics) IN THE PHYSICS DEPARTMENT—The Dean, Guy's Hospital Medical School, London, S.E.1 (May 9).

ASSISTANT LECTURER (with special qualifications in pure mathematics) IN MATHEMATICS—The Registrar (Room 29, O.R.B.), The University, Reading (May 9).

RESEARCH FELLOW IN THE CHEMISTRY DEPARTMENT, for work on fundamental aspects of heterogeneous catalysis, in collaboration with Dr. G. C. Bond—The Professor of Chemistry, The University, Hull (May 9).

EDWARD A. DEEDS FELLOW (man or woman), for research in Engineering, Metallurgy, Chemistry, Physics, Mathematics or any cognate subject—Joint Clerk to the University Court, Queen's College (University of St. Andrews), Dundee (May 10).

LECTURER/SENIOR LECTURER IN NUTRITION at the University of New England, Australia, to undertake teaching and research in Livestock Nutrition in the Department of Nutrition and Chemical Pathology—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, May 13).

BOWMAN CHAIR OF PHYSIOLOGY—The Registrar, The University, Edgbaston, Birmingham 15 (May 14).

CHAMBER OF MANUFACTURES CHAIR OF PHYSICS in the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (May 15).

LECTURER OR ASSISTANT LECTURER IN BOTANY at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (May 15).

LECTURER (with special qualifications and experience in the food industry (other than the dairy industry) or in biochemical engineering) IN FOOD SCIENCE—The Registrar (Room 22, O.R.B.), The University, Reading (May 16).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/N (May 21).

LECTURER (graduate in engineering, with an interest in the application to agricultural operations of electronic and other control methods) IN FARM MECHANIZATION (ELECTRICAL ENGINEERING)—The Registrar (Room 29, O.R.B.), The University, Reading (May 21).

ELDER CHAIR OF PHYSICS in the University of Adelaide—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, May 31).

READER or SENIOR LECTURER (with appropriate qualifications and experience needed to take charge of the newly established Statistics Unit) IN STATISTICS at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong and London, May 31).

ASSISTANT LECTURER (with a Ph.D. in microbiology and research experience in this field) IN THE DEPARTMENT OF INDUSTRIAL MICROBIOLOGY—Prof. E. Küster, Department of Industrial Microbiology, University College, Ardmore House, Stillorgan Road, Dublin.

ASTRONOMER (Senior Assistant) (with main interest in stellar astronomy)—The Director, Armagh Observatory, Armagh, Northern Ireland.

DEMONSTRATOR IN BIOCHEMISTRY—The Administrator, Department of Biochemistry, South Parks Road, Oxford.

DIRECTOR (age 40–45, with a good degree or diploma in geology plus wide experience) OF THE PAKISTAN GEOLOGICAL SURVEY—The Recruitment Officer, Pakistan High Commission, 39 Lowndes Square, London, S.W.1.

INSTRUCTORS or ASSISTANT PROFESSORS (well-qualified candidates) IN THE DEPARTMENT OF CHEMISTRY—Prof. C. A. McDowell, Department of Chemistry, University of British Columbia, Vancouver 8, B.C., Canada.

LECTURERS IN THE MATHEMATICS DEPARTMENT—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

RESEARCH ASSISTANT (chemistry graduate), to continue and extend a research project into the kinetics and mechanism of the reaction of sulphur monochloride with olefines—Head, National College of Rubber Technology, Northern Polytechnic, Holloway, London, N.7.

RESEARCH FELLOW (Fellow of the Library Association)—The Secretary, Library Association, Chaucer House, Malet Place, London, W.C.1.

SENIOR SCIENTIST (with experience in the use of radioactive isotopes for medical research), to organize an active research group and initiate a programme of fundamental research including the development of new clinical procedures—Dr. H. G. Thode, Vice-President, McMaster University, Hamilton, Ontario, Canada.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Coke and Chemistry, U.S.S.R., 1959, No. 8. (Organ of the State Scientific-Technical Committee of the Council of Ministers of the U.S.S.R.) (An English version of the monthly journal *Koks i Khimiya*, prepared by the Coal Tar Research Association, in collaboration with the British Coke Research Association.) Pp. 59. Annual subscription 105*s*. Single issues 20*s*. (Gomersal, Leeds: The Coal Tar Research Association, 1959.) [32

Department of Scientific and Industrial Research: Road Research Laboratory. Road Research Technical Paper No. 44: A Study of the Granulators used in the Production of Roadmaking Aggregates. By F. A. Shergold. Pp. v+70+8 plates. (London: H.M. Stationery Office, 1959.) 5*s*. 6*d*. net. [32

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Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 1007, Vol. 252 (28 January 1960): The Absorption Spectrum of the Free NCO Radical. By R. N. Dixon. Pp. 165–192. 12*s*. No. 1008, Vol. 252 (28 January 1960): X-ray Studies of some Carbonized Coals. By R. Diamond. Pp. 193–223. 10*s*. (London: Royal Society, 1960.) [32

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Production Engineering Research Association of Great Britain. PERA Report No. 13: Tapping. Part 1: Relationship between Core Hole Size, Tapping Torque and Thread Strength. Pp. 12. (Melton Mowbray: Production Engineering Research Association of Great Britain, 1959.) [42

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Biochemical and Biophysical Research Communications, Vol. 1, No. (July 1959). Published monthly. Pp. 44. Subscription rates: volume, 6 issues)—Institutional subscribers 12 dollars; 85*s*. Private subscribers certifying that the volume will be for personal u only, 6.50 dollars; 46*s*. (New York and London: Academic Pres Inc., 1959.)

Publications of the United States Naval Observatory. Second Serie Vol. 17, Part 6: Polarization of Starlight in the Galaxy. By Joh S. Hall. Pp. iii+275–342+plates 4–6. (Washington, D.C.: Gover ment Printing Office, 1958.)

U.S. Department of Health, Education and Welfare: Public Heal Service. National Library of Medicine. *Index Medicus*, Vol. 1, No. (January 1960). (Formerly *Current List of Medical Literature*.) P 282. (Washington, D.C.: Government Printing Office, 1960.) 2. dollars.

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